The American Journal of Medicine

SYMBOL OF DISTINGUISHED JOURNALISM

The American Journal of Medicine

Editor: ALEXANDER B. GUTMAN, M.D.
Professor of Medicine
COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK
DIRECTOR, DEPARTMENT OF MEDICINE, THE MOUNT SINAI HOSPITAL, NEW YORK

Assistant Editors: MORTIMER E. BADER, M.D. AND RICHARD A. BADER, M.D.
THE MOUNT SINAI HOSPITAL, NEW YORK

ADVISORY BOARD

DAVID P. BARR, M.D.
Professor Emeritus of Medicine
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK

ARTHUR L. BLOOMFIELD, M.D.
Professor Emeritus of Medicine
SCHOOL OF MEDICINE, STANFORD UNIVERSITY
SAN FRANCISCO

A. MCGEHEE HARVEY, M.D.
Professor of Medicine
JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE
BALTIMORE

WALTER L. PALMER, M.D.
Professor of Medicine
UNIVERSITY OF CHICAGO, SCHOOL OF MEDICINE
CHICAGO

ASSOCIATE EDITORS

S. HOWARD ARMSTRONG, JR., M.D., *Chicago*
J. RUSSELL ELKINTON, M.D., *Philadelphia*
PETER H. FORSHAM, M.D., *San Francisco*
ROBERT M. KARK, M.D., *Chicago*
WILLIAM S. MCCANN, M.D., *Rochester, N. Y.*
GEORGE R. MENEELY, M.D., *Nashville*
CARL V. MOORE, M.D., *St. Louis*
JACK D. MYERS, M.D., *Pittsburgh*
ROBERT E. OLSON, M.D., *Pittsburgh*
DEWITT STETTEN, JR., M.D., *Bethesda*
JOHN V. TAGGART, M.D., *New York*
GEORGE W. THORN, M.D., *Boston*

THE AMERICAN JOURNAL OF MEDICINE is published monthly by The American Journal of Medicine, Inc., Executive and Editorial Offices, 11 East 36th Street, New York 16, N. Y. May 1960, Volume 28, No. 5. Copyright © 1960 by The American Journal of Medicine, Inc., subsidiary of The Reuben H. Donnelley Corporation. No part of the contents of this publication may be reproduced or distributed without the express written consent of the publishers. Publication office, York, Pa. Second-class postage paid at York, Pa.

SUBSCRIPTIONS: United States $12.00 a year; Canada $13.00; Foreign $16.00
SINGLE COPIES: Regular Issues $2.00; Symposium and Special Issues $4.00

MAIL CHANGES OF ADDRESS AND SUBSCRIPTION ORDERS TO: The American Journal of Medicine, 11 East 36th Street, New York 16, N. Y. Change of address must reach us one month in advance.

MANUSCRIPTS: All manuscripts should be typewritten double space and submitted to the Editorial Office of The American Journal of Medicine, 11 East 36th Street, New York 16, N. Y. The top should be indicated on the back of each photograph. Reference style: Hall, C. A. Erythrocyte dynamics in liver disease. Am. J. Med., 28: 541, 1960.

PLINY A. PORTER—Publisher
JOHN J. MARTIN—Assistant Publisher

after a myocardial infarction
and throughout the course
of postcoronary convalescence...

Peritrate helps establish and sustain collateral circulation

1. improves coronary blood flow with no significant drop in blood pressure or increase in pulse rate
2. helps support natural healing and repair
3. helps reduce myocardial damage
4. smooth onset of action minimizes nitrate headache

basic therapy in coronary artery disease

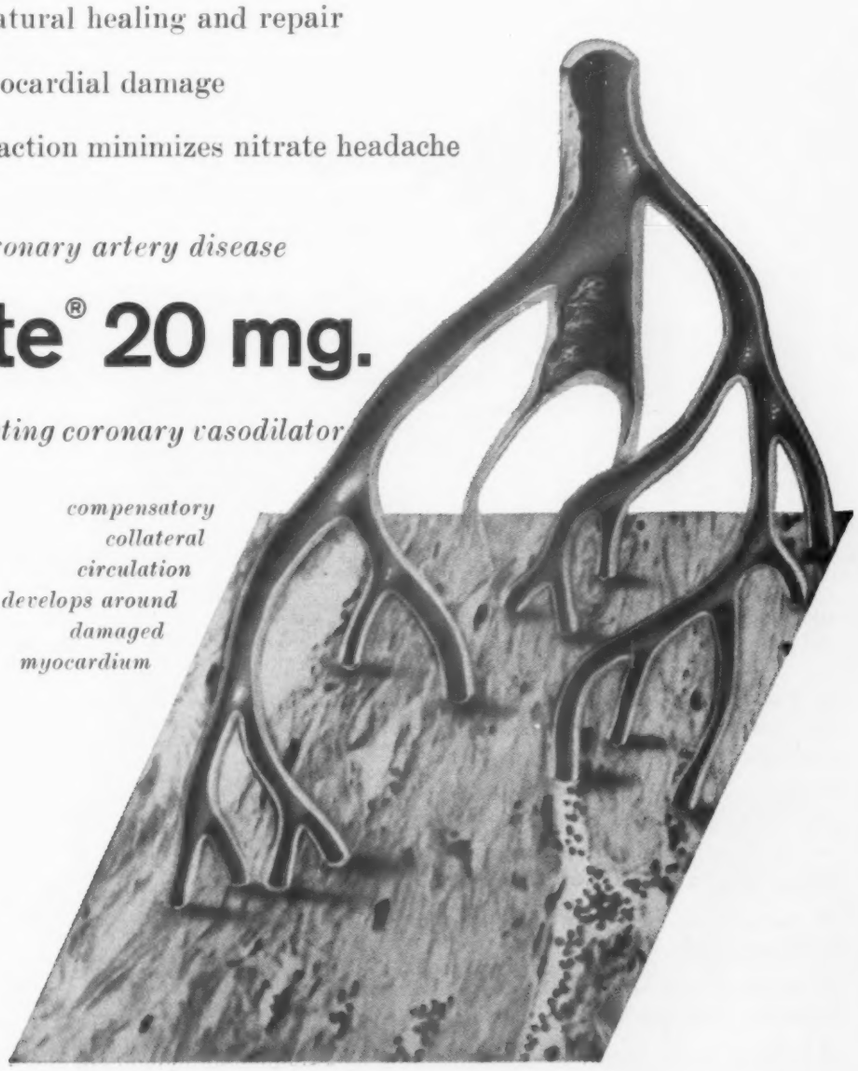
Peritrate® 20 mg.

brand of pentaerythritol tetranitrate

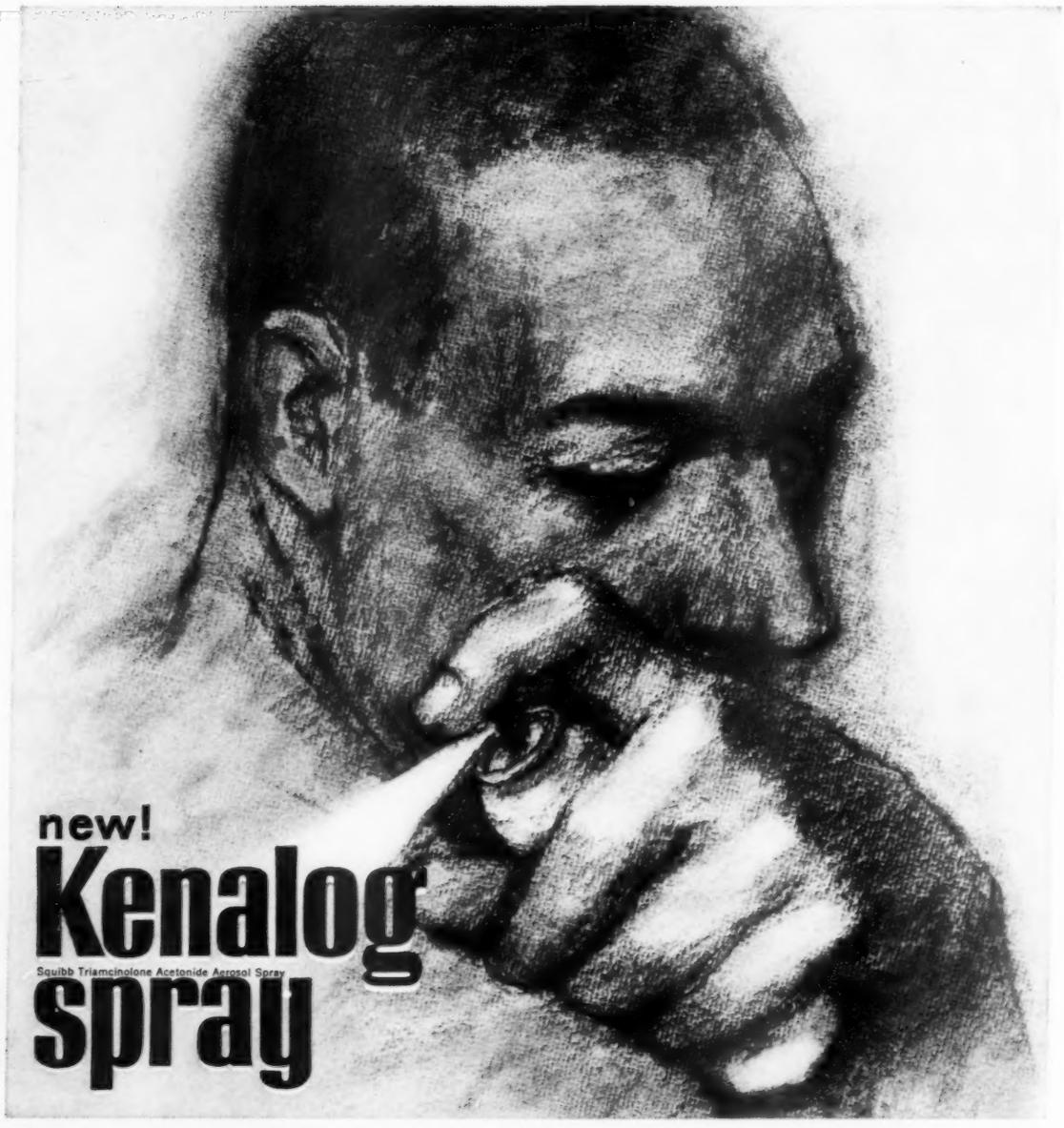
the selective, long-acting coronary vasodilator

WARNER
CHILCOTT
MORRIS PLAINS, N.J.

GP-02



compensatory collateral circulation develops around damaged myocardium



superior topical corticosteroid

In convenient aerosol spray—for the treatment of dermatoses, particularly in out-of-reach problem areas—a fresh approach to therapy

- the superior anti-inflammatory effect of Kenalog Spray[1-7] provides anti-inflammatory, antiallergic, and antipruritic relief in acute, exudative, weeping lesions, even in extensive, out-of-reach problem areas.
- minimal local irritation and less chance of local contamination.
- metabolic studies show electrolyte disturbance does not occur when Kenalog is applied topically.[1, 4, 5]
- easy to apply, gives broad, even coverage, and permits observation of lesions as they heal.

Indications: Atopic dermatitis, contact dermatitis, eczematous dermatitis, neurodermatitis, seborrheic dermatitis, insect bites, pruritus ani and vulvae, lichen simplex chronicus, exfoliative dermatitis, stasis dermatitis, nummular eczema, sunburn.

Dosage: Apply the spray to the affected areas from a distance of 3 to 6 inches, t.i.d. or q.i.d. A 3-second spray (delivering approximately 0.1 mg. of triamcinolone acetonide) covers an area about the size of the hand. *Cover the eyes when using Kenalog Spray on or near the face.*

Supply: Kenalog Spray in 50 and 150 Gm. containers of 3.3 mg. and 10 mg. triamcinolone acetonide respectively. Also available—Kenalog Cream (0.1%), Kenalog Ointment (0.1%) and Kenalog Lotion (0.1%).

References: 1. Reports to the Squibb Institute for Medical Research. 2. Howell, C. M.: Squibb Clin. Res. Notes *1*:5 (Oct.) 1958. 3. Goodman, J. J.: Squibb Clin. Res. Notes *1*:1 (Oct.) 1958. 4. Smith, J. G., Jr.; Zawisza, R. J., and Blank, H.: Squibb Clin. Res. Notes *1*:6 (Oct.) 1958. 5. Fitzpatrick, T. B.; Crowe, F. W., and Walker, S. A.: Squibb Clin. Res. Notes *1*:1 (Oct.) 1958. 6. Lerner, A. B.: Squibb Clin. Res. Notes *1*:2 (Oct.) 1958. 7. Robinson, R. C. V.: Bull. School of Med., U. Maryland *43*:54 (July) 1958.

*KENALOG® IS A SQUIBB TRADEMARK.

SQUIBB
Squibb Quality—
the Priceless Ingredient

The American Journal of Medicine

Vol. XXVIII MAY 1960 No. 5

CONTENTS

Symposium on Leukemia

Introduction . . . . . JOHN S. LAWRENCE 671

Radiation Leukemogenesis: An Analysis of the Problem
E. P. CRONKITE, WILLIAM MOLONEY AND V. P. BOND 673

The Virus Theory of Leukemia . . JEROME T. SYVERTON AND JOHN D. ROSS 683

The Metabolism of the Leukemic Leukocyte . . . . . WILLIAM N. VALENTINE 699

The Physiology of Granulocytic Cells in Normal and Leukemic States
C. G. CRADDOCK, JR. 711

Purine and Pyrimidine Excretion in Normal and Leukemic Subjects
WILLIAM S. ADAMS, FRANCES DAVIS AND MISAE NAKATANI 726

Mechanisms of Drug Action in Leukemia . . . . . IRWIN H. KRAKOFF 735

The Anemia of Leukemia
STANLEY B. TROUP, SCOTT N. SWISHER AND LAWRENCE E. YOUNG 751

Clinical Manifestations of Leukemia. Recent Contributions
BARRY FIRKIN AND CARL V. MOORE 764

The Clinical Management of Leukemia
ARTHUR HAUT, MAXWELL M. WINTROBE AND G. E. CARTWRIGHT 777

Attempts at Transplantation of Human Bone Marrow in Patients with Acute Leukemia and Other Marrow Depletion Disorders
FARID I. HAURANI, EVALYN REPPLINGER AND L. M. TOCANTINS 794

Contents continued on page 5

ALEVAIRE® aerosol in the home

in bronchiectasis–

*"Thick, yellow, solid sputum which had been expectorated with difficulty became thin, colorless and liquid sputum which was expectorated with ease and gradually diminished in volume. Labored breathing and insomnia, . . . soon were replaced by easy respiration and ability to enjoy normal restful sleep."**

CASE REPORT

A typical Alevaire case history—C. S., 31 year old male with bronchiectasis and sinusitis, had had pneumonia six times. He had a continuous thick purulent postnasal drip and thick, yellowish green sputum; he expectorated at least a cupful of sputum each morning on arising. The patient was weak and debilitated, with chills and low grade fever. Bronchograms revealed advanced bronchiectasis. Antibiotics, postural drainage and expectorant cough mixtures had not helped.

Alevaire therapy was begun with one hour of direct nasal inhalation every day. After the first treatment the patient expectorated a large amount of sputum and definitely breathed easier. The nasal passages began to open, and with subsequent treatments both the sinusitis and the bronchiectasis improved. He began to breathe easier through the nose and to expel bronchial secretions more readily. His appetite improved and he felt stronger.

At the end of fourteen days he was almost completely symptom free. Alevaire was continued each night for short periods at bedtime, and the patient remained completely free of symptoms except for a light morning expectoration.

*Miller, J.B., et al.: Ann. Allergy, 12:611, Sept.-Oct., 1954.

ALEVAIRE

has been dramatically effective in:

- neonatal asphyxia (due to inhalation of amniotic fluid, mucus obstruction, atelectasis)
- croup • laryngitis • tracheobronchitis
- pertussis • pneumonia • bronchial asthma
- emphysema • bronchiectasis • lung abscess
- pneumoconiosis • smoke, kerosene poisoning
- poliomyelitis (respiratory complications)
- routine oxygen therapy • tracheotomy
- prevention of postoperative pulmonary complications

Alevaire is supplied in bottles of 60 cc. for intermittent therapy and in bottles of 500 cc. for continuous inhalation therapy.

Winthrop LABORATORIES
NEW YORK 18, N. Y.

Alevaire, trademark reg. U.S. Pat. Off.

CONTENTS continued – May 1960

VOLUME TWENTY-EIGHT NUMBER FIVE

Clinical Studies

Primary Hemorrhagic Thrombocythemia
FARUK L. OZER, WAYNE E. TRUAX, DAVID C. MIESCH AND WILLIAM C. LEVIN 807

Marked thrombocytosis not associated with spenectomy, acute hemorrhage or extensive tissue necrosis ordinarily is an accompaniment or antecedent of overt chronic granulocytic leukemia or polycythemia vera. There is some indication, however, that it may occur independently as a primary disease. In this instance it is part of a panmyelosis expressed principally by increased platelet formation, i.e., one of the myeloproliferative syndromes, often overlapping with myeloid metaplasia. The disease pattern is then characterized by recurrent thrombohemorrhagic phenomena, enlargement of the spleen and often of the liver, evidences of excessive platelet production in the bone marrow and peripheral blood, and moderate erythrocytosis and granulocytosis. Such a classification admittedly is controversial, but recognition of the syndrome nevertheless is important because appropriate therapy usually prolongs life.

A Simplified Closed Circuit Helium Dilution Method for the Determination of the Residual Volume of the Lungs
GEORGE R. MENEELY, CON O. T. BALL, ROSS C. KORY, JAMES J. CALLAWAY, JOSEPH MERRILL, R. E. MABE, DAN C. ROEHM AND NOLAN L. KALTREIDER 824

Determination of the residual volume of the lungs has become increasingly important as its usefulness in the diagnosis of pulmonary dysfunction and in assessment of medical and surgical therapy has grown more apparent. However, the methodological complexities of more precise measurements, particularly by dilution methods employing helium, have acted as a deterrent to wider application. This paper offers a simplified rapid version of the helium dilution method. Sources of error are carefully analyzed and the accuracy of the method established.

Mucormycosis. Two Cases of Disseminated Disease with Cultural Identification of Rhizopus; Review of Literature
RAYMOND A. MCBRIDE, JOSEPH M. CORSON AND GUSTAVE J. DAMMIN 832

Mucormycosis is turning up more frequently in recent years and not only as a terminal meningoencephalitic infection in diabetic patients, but also in nondiabetic subjects. The present study summarizes fifty-five published cases of mucormycosis, including two new cases herein recorded, and stresses occurrence of pulmonary and disseminated involvement by this fungal infection in non-diabetic subjects, notably those with leukemia or lymphoma, or any other debilitating disease. While usually fatal, recovery from mucormycosis is possible, hence diagnosis during life is important for institution of appropriate therapeutic measures.

Contents continued on page 7

for the tense and nervous patient

proven effective and outstandingly safe

- simple dosage schedule produces rapid, predictable tranquilization without unexpected excitation
- no cumulative effects, thus no need for difficult dosage readjustments
- does not produce ataxia, change in appetite or libido
- no danger of hypotension, depression, Parkinson-like reactions, jaundice or agranulocytosis
- does not impair mental efficiency or normal behavior

Usual dosage: One or two 400 mg. tablets t.i.d.
Supplied: 400 mg. scored tablets, 200 mg. sugar-coated tablets; or as MEPROTABS*—400 mg. *unmarked*, coated tablets.
*TRADE-MARK

Miltown®
meprobamate (Wallace)

WALLACE LABORATORIES / *New Brunswick, N. J.*

CM-1901

CONTENTS continued–May 1960

VOLUME TWENTY-EIGHT NUMBER FIVE

Case Reports

"Kwashiorkor-Like" Syndrome, Associated with Burning Feet Syndrome, in an Adult Male . . . . CHARLES W. SILVERBLATT AND HARVEY E. BROWN, JR. 847

A case of unusual interest.

Coronary Embolism
GERAINT T. GRIFFITH, JOHN TURNER SHARP AND DAVID G. GREENE 855

An interesting report.

Rupture of an Aneurysm of the Sinus of Valsalva
EUGENE J. LIPPSCHUTZ AND LORING W. WOOD 859

A well studied and instructive case of rupture of the posterior (non-coronary) sinus of Valsalva into the right atrium. The diagnosis, suspected clinically, was confirmed by cardiac catheterization and retrograde aortography. The shunt was successfully closed surgically.

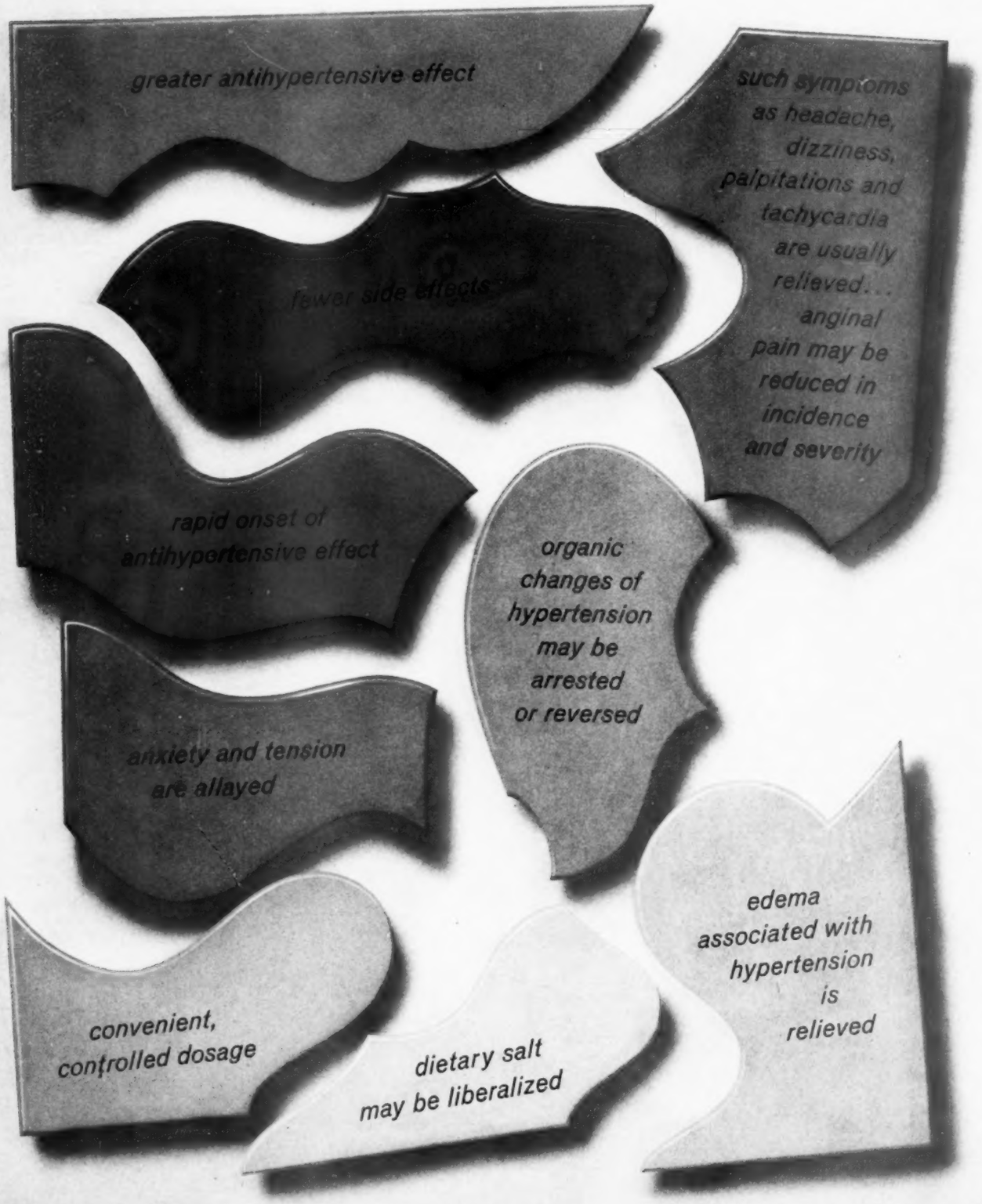
Maffucci's Syndrome (Dyschondroplasia with Hemangiomas)
JEROME L. JOHNSON, JAMES R. WEBSTER, JR. AND H. IVAN SIPPY 864

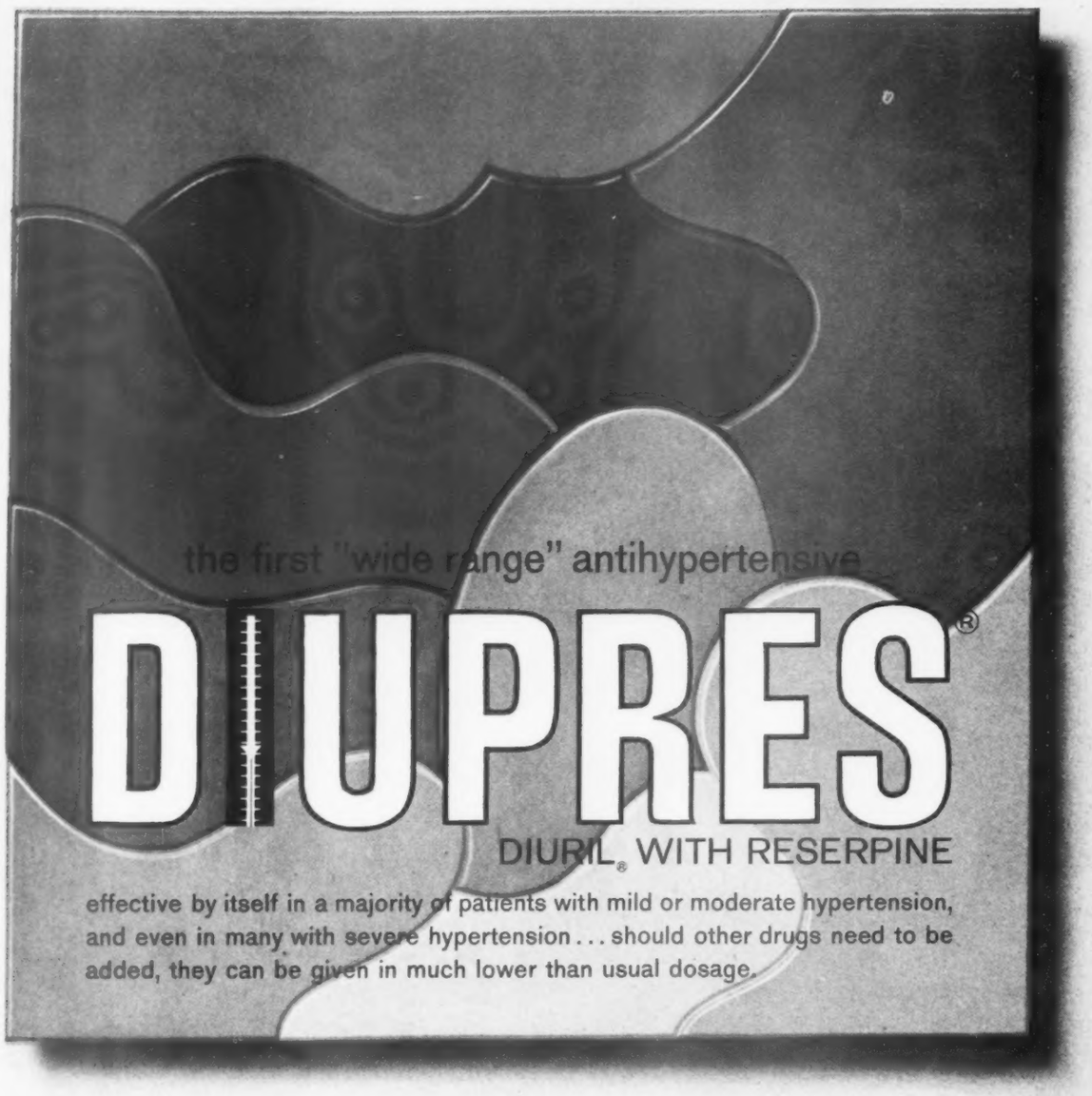
A syndrome worth noting.

Advertising Index on Pages 161 and 162

Change of address must reach us one month preceding month of issue.

here are some of the ways hypertensive patients benefit when you prescribe **DIUPRES**


greater antihypertensive effect
such symptoms as headache, dizziness, palpitations and tachycardia are usually relieved... anginal pain may be reduced in incidence and severity
fewer side effects
rapid onset of antihypertensive effect
organic changes of hypertension may be arrested or reversed
anxiety and tension are allayed
edema associated with hypertension is relieved
convenient, controlled dosage
dietary salt may be liberalized


the first "wide range" antihypertensive
DIUPRES®
DIURIL® WITH RESERPINE
effective by itself in a majority of patients with mild or moderate hypertension, and even in many with severe hypertension . . . should other drugs need to be added, they can be given in much lower than usual dosage.

DIUPRES-250

250 mg. DIURIL (chlorothiazide),
0.125 mg. reserpine per tablet.
One tablet one to four times a day.

DIUPRES-500

500 mg. DIURIL (chlorothiazide),
0.125 mg. reserpine per tablet.
One tablet one to three times a day.

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.

DIUPRES and DIURIL are trademarks of Merck & Co., Inc.

MSD MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

AN AMES CLINIQUICK®

CLINICAL BRIEFS FOR MODERN PRACTICE

WHY IS DIABETES IN INFANTS SO DIFFICULT TO DIAGNOSE?

Because of the infrequency of the disease in this age group, its sudden onset, the profusion of inconsistent presenting symptoms, and because the accompanying symptoms of anorexia and vomiting are also characteristic symptoms of many other ills of infancy.

Source: Traisman, H. S.; Boehm, J. J., and Newcomb, A. L.: Diabetes *8*:289, 1959.

for those pediatric puzzlers..."A routine urinalysis and blood sugar should be done whenever the possibility of diagnosing diabetes is entertained."*

DIABETES MELLITUS AT AGES 1 TO 5

Order of Frequency of Presenting Symptoms in 110 Patients

Symptoms	No. of Patients	Per cent of total group
Polyuria	93	84.5
Polydipsia	89	81.0
Weight loss	47	42.7
Polyphagia	28	25.4
Anorexia	16	14.5
Lethargy	14	12.7
Enuresis	7	6.4
Vomiting	5	4.5
Irritability	3	2.7
"Craving for sweets"	3	2.7
"Sticky diaper"	3	2.7
"Strong odor to urine"	2	1.8
Glycosuria	2	1.8
Hypoglycemia	2	1.8
Personality change	1	0.9
Boils	1	0.9
Headache	1	0.9
Abdominal cramps	1	0.9

Adapted from Traisman, H. S.; Boehm, J. J., and Newcomb, A. L.*

the standardized urine-sugar test for reliable quantitative estimations

AMES COMPANY, INC. Elkhart, Indiana · Toronto, Canada

COLOR-CALIBRATED CLINITEST®

BRAND Reagent Tablets

84060

- full-color calibration, clear-cut color changes
- established "plus" system covers entire critical range
- standard blue-to-orange spectrum
- standardized, laboratory-controlled color scale
- "urine-sugar profile" graph for closer control

the **"Stylish Stout"**
who'd like a
"Junior Miss"
figure

To help her slim down to "bathing beauty" size—for her health's sake as well as for her social need—

SYNDROX®

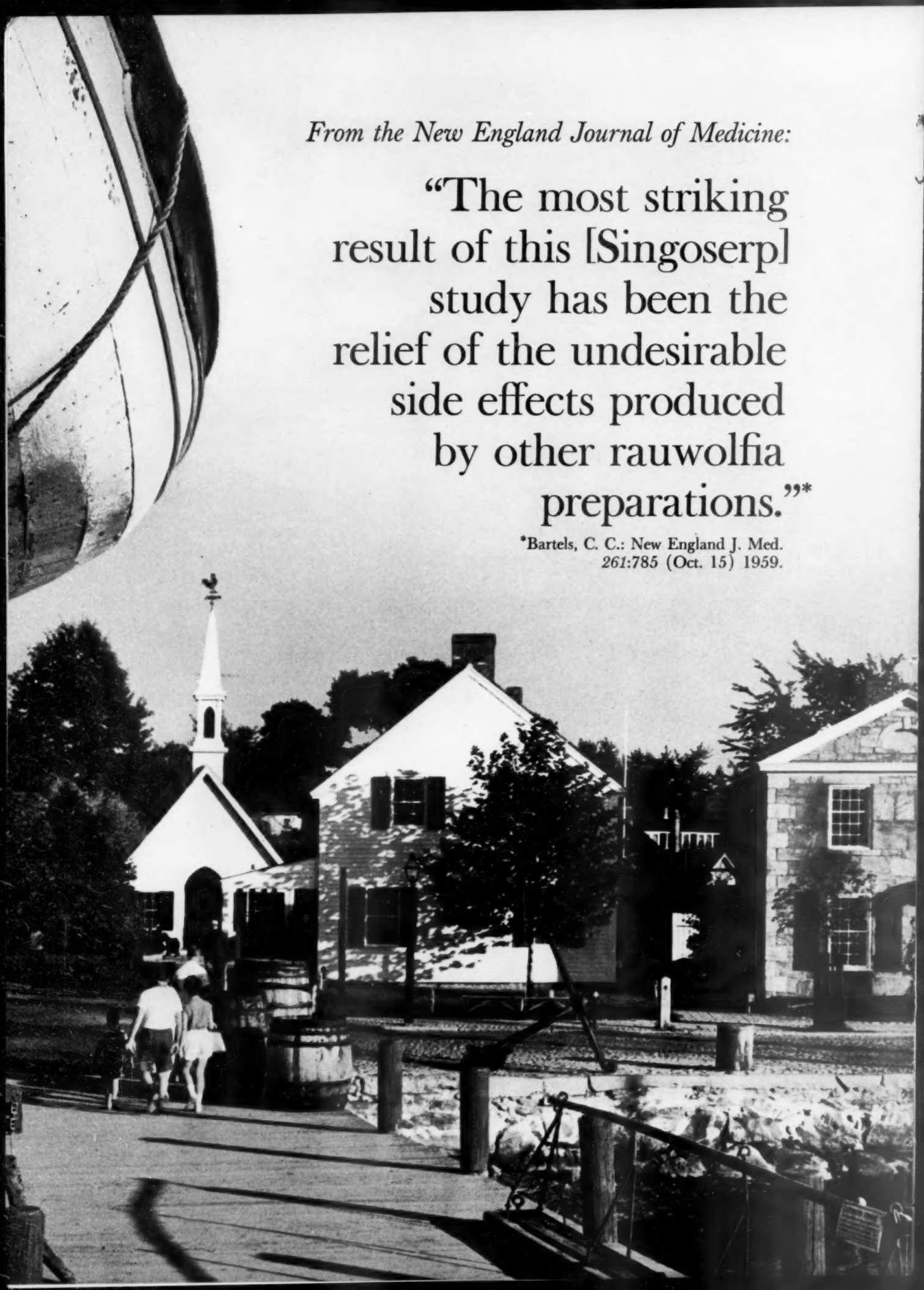
methamphetamine hydrochloride

- controls the desire for constant nibbling—suppresses appetite
- takes the mind off food by encouraging activity and a brighter viewpoint
- Dose for appetite suppression: ½ to 1 tab. or tsp. before meals.

SYNDROX TABLETS (5 mg.)
ELIXIR (5 mg. per 5 cc.)

McNEIL

McNEIL LABORATORIES, INC. PHILADELPHIA 32, PA.



From the New England Journal of Medicine:

"The most striking result of this [Singoserp] study has been the relief of the undesirable side effects produced by other rauwolfia preparations."*

*Bartels, C. C.: New England J. Med. *261*:785 (Oct. 15) 1959.

Results you can confirm in your practice:

If you have ever treated a patient for hypertension with a rauwolfia preparation, you are no doubt familiar with the possible side effects of such medication — fatigue or lethargy, depression, nasal congestion, gastrointestinal distress, etc. But with Singoserp you can expect these improved results: "... when syrosingopine was substituted for the rauwolfia product, the blood-pressure response was equally good. In 24 cases syrosingopine was substituted for the rauwolfia product because of 26 troublesome side effects; these symptoms were relieved in all but 3 patients."*

Side Effects	Incidence with Prior Rauwolfia Agent	Incidence with Singoserp
Depression	11	1
Lethargy or fatigue	5	0
Nasal congestion	7	0
Gastrointestinal disturbances	2	2
Conjunctivitis	1	0

(Adapted from Bartels*)

many hypertensive patients prefer

Singoserp®

(syrosingopine CIBA)

because it lowers their blood pressure without rauwolfia side effects

SUPPLIED: *Tablets,* 1 mg. (white, scored); bottles of 100. 2/2785HK

CIBA
SUMMIT, NEW JERSEY

'round-the-clock relief
of Duodenal Ulcers
and other G.I. disturbances

with

daricon®

oxyphencyclimine HCl, 10 mg.

b.i.d.

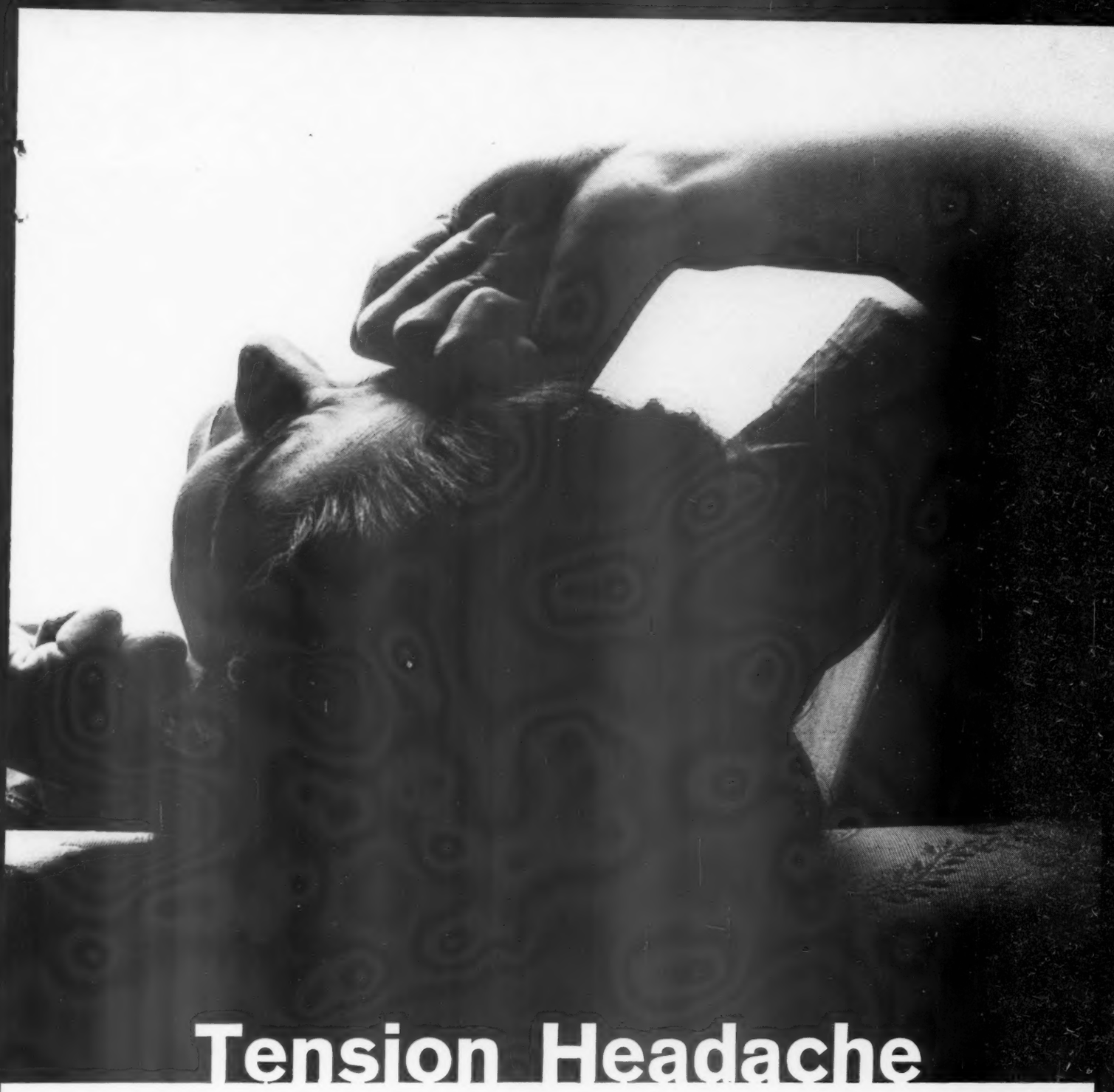
"Good symptomatic responses were seen in 91 of 96 [patients] treated for periods up to one year with average doses of 10 mg. twice daily."

"[Daricon] appears to be a valuable agent . . . for day-to-day maintenance of all peptic ulcer patients."

Winkelstein, A.: Am. J. Gastroenterol. *32*:66-70 (July) 1959.

Additional information is available on request from the Medical Department, Pfizer Laboratories, Brooklyn 6, N. Y.

Pfizer Science for the world's well-being™



Tension Headache

Relief without Analgesics

"Meprobamate was found of considerable value in reducing the tension component of chronic headache, and thus decreasing the frequency and severity of attacks. No intolerance, dependence, or side effects were noted. . . ."
Blumenthal, L.S., and Fuchs, M.: Am. Pract. & Digest Treat. 9:1121 (July) 1958.

An extensive bibliography attests to the striking effectiveness and noteworthy safety of EQUANIL (meprobamate, Wyeth) in a variety of tension and anxiety states. EQUANIL possesses specific action; pharmacological action is not diffuse. Rapid metabolism precludes cumulative effects.

Although rare, allergic reactions may occur; excessive dosage should be avoided in all patients. For further information on prescribing and administering EQUANIL see descriptive literature, available on request.

Wyeth Laboratories Philadelphia 1, Pa.

Equanil®
Meprobamate, Wyeth

ANNIVERSARY 1860 1960 Wyeth

A Century of Service to Medicine

highly effective,

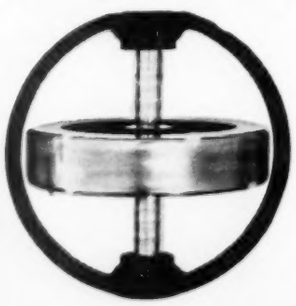
in respiratory allergies...

unsurpassed for total corticosteroid benefits

Aris

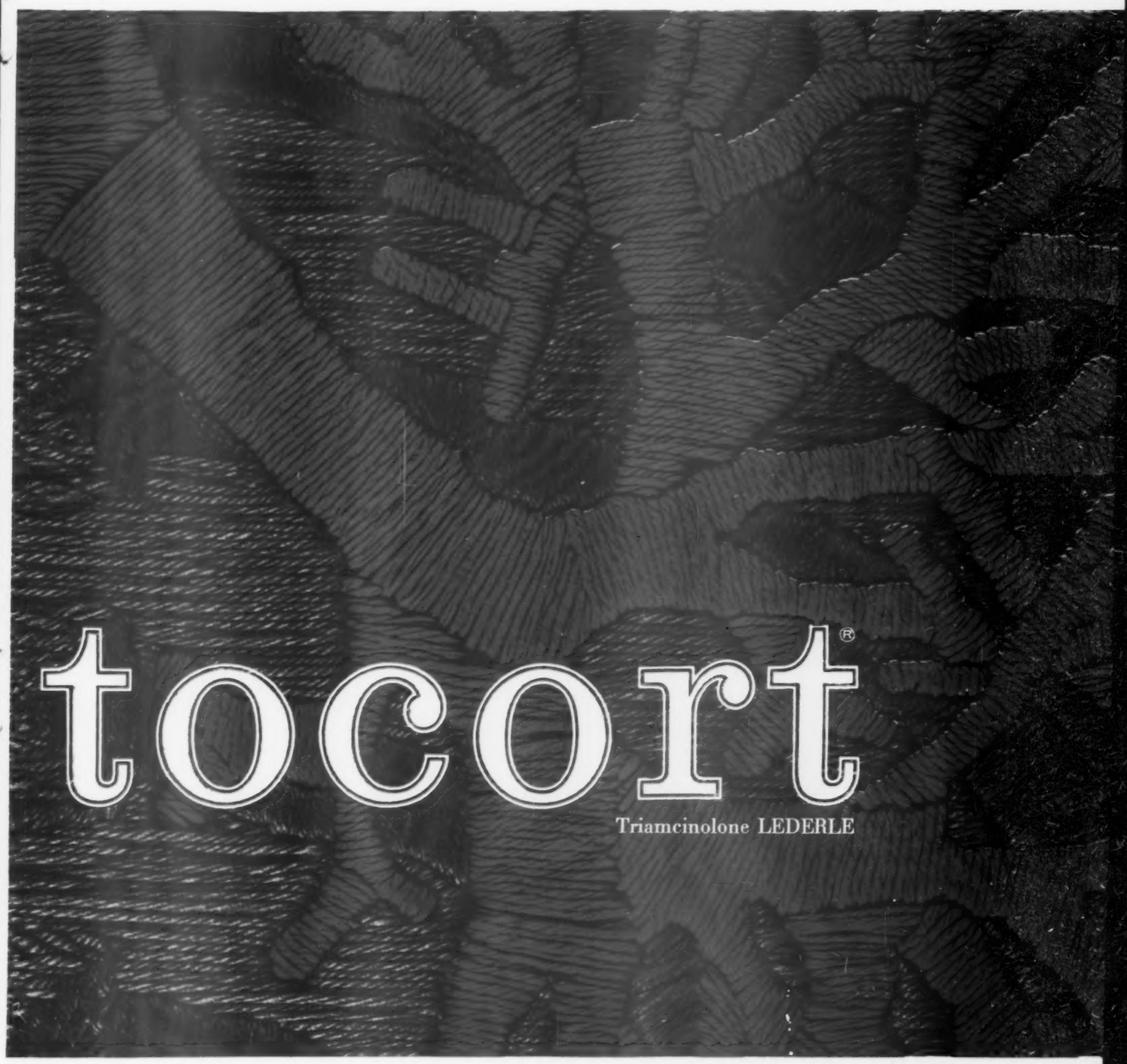
Substantiated by published reports of leading clinicians:

- effective control of inflammatory and allergic symptoms[1-8]



- biochemical and psychic balance disturbance minimal[1, 4, 5, 8-18]

well-tolerated control



A Promise Fulfilled

All corticosteroids provide symptomatic control in rheumatoid arthritis, bronchial asthma and inflammatory dermatoses. They *differ* in the frequency and severity of side effects. Introduced in 1958, ARISTOCORT Triamcinolone bore the promise of high efficacy and relative safety

Physicians today recognize that the promise has been fulfilled... as evidenced by the high rate of refilled ARISTOCORT prescriptions.

List of References 1-18 supplied on request.

Precautions: With ARISTOCORT all precautions traditional to corticosteroid therapy should be observed. Dosage should always be carefully adjusted to the smallest amount which will suppress symptoms.

Supplied:
1 mg. scored tablets (yellow)
2 mg. scored tablets (pink)
4 mg. scored tablets (white)
16 mg. scored tablets (white)

Lederle LEDERLE LABORATORIES, A Division of AMERICAN CYANAMID COMPANY, Pearl River, N.Y.

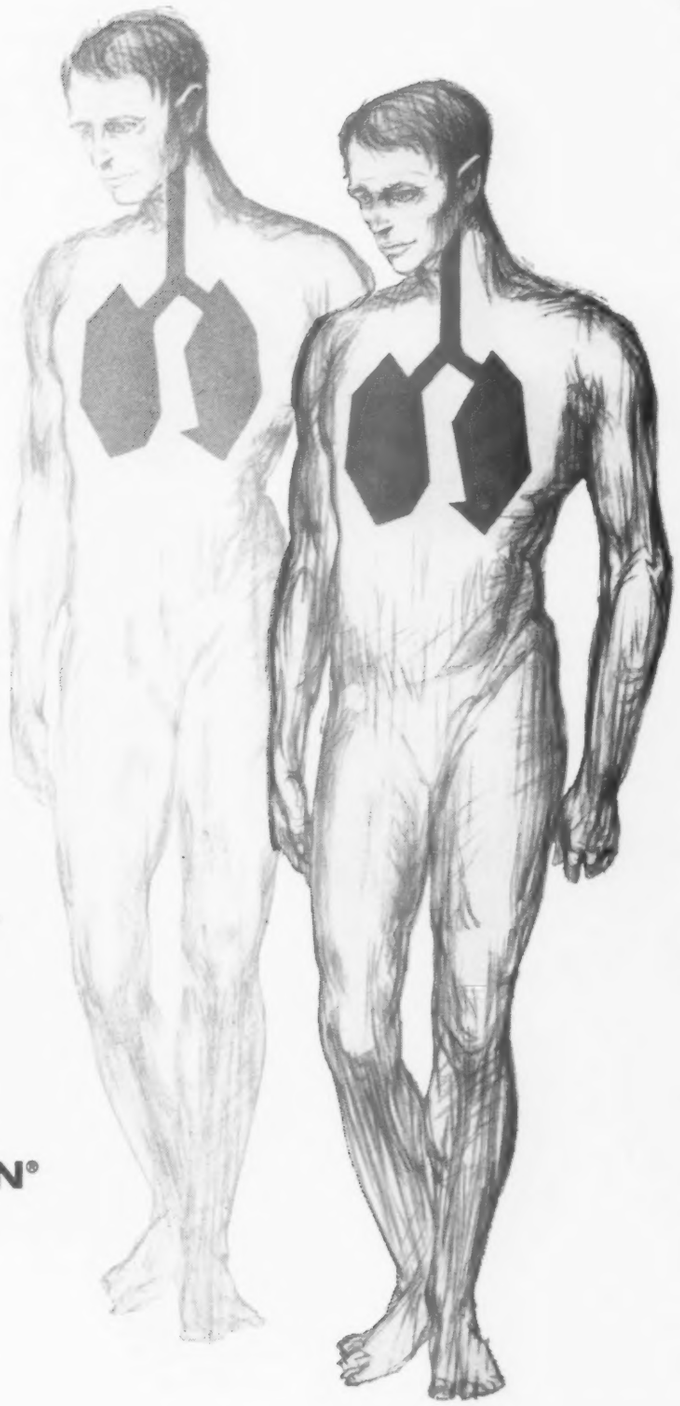
potentiated therapy for mild to moderate hypertension

Consider the benefits of Singoserp-Esidrix if it's mild to moderate hypertension (especially if edema is a complicating symptom). Singoserp, a man-made analog of reserpine, lowers blood pressure but seems to cause fewer side effects than natural rauwolfia compounds. When Singoserp is potentiated by Esidrix, blood pressure is lowered more effectively than with single-drug therapy. SUPPLIED IN TWO STRENGTHS: Singoserp-Esidrix *Tablets #2* (each containing 1 mg. Singoserp and 25 mg. Esidrix) and Singoserp-Esidrix *Tablets #1* (each containing 0.5 mg. Singoserp and 25 mg. Esidrix). Complete information available on request.

Singoserp®-Esidrix®
(syrosingopine® and hydrochlorothiazide CIBA)

CIBA
SUMMIT · NEW JERSEY

2/2764MB



the margin of difference in respiratory tract infections

COSA-TERRAMYCIN®

oxytetracycline with glucosamine

CAPSULES

The greater effectiveness, proven safety and outstanding toleration of Terramycin provide a margin of difference for swift response and uncomplicated recovery.

This margin is further extended by convenient, economical, ready-to-use Terramycin Intramuscular Solution followed by oral Cosa-Terramycin – the compatible, coordinated course of broad-spectrum therapy worthy of consideration for your next patient with a respiratory infection.

Pfizer *Science for the world's well-being™*

Pfizer Laboratories, Div., Chas. Pfizer & Co., Inc.
Brooklyn 6, N. Y.

Supply: Cosa-Terramycin Capsules—250 mg. and 125 mg. New Cosa-Terrabon* Oral Suspension—125 mg./5 cc. (tsp.), preconstituted, fruit flavored, bottles of 2 oz. and 1 pint. New Cosa-Terrabon Pediatric Drops—100 mg./cc. (5 mg./drop), preconstituted, fruit flavored, 10 cc. bottle with calibrated plastic dropper. Terramycin Intramuscular Solution†—ampules of 100 mg./2 cc. and 250 mg./2 cc.

Terramycin is also available in a variety of topical and local forms to meet specific therapeutic requirements.

*Trademark
†Contains 2% Xylocaine® (lidocaine), registered trademark of Astra Pharmaceutical Products, Inc.

NO SPRAIN, NO STRAIN, OR LOW BACK PAIN

can resist the rapid relaxant relief of

RELA™

CARISOPRODOL

RELA—SCHERING'S MYOGESIC[x]
RELAXES MUSCLE TENSION
FOR MORE ADEPT MANAGEMENT
OF BOTH SPASM AND ITS PAIN

Rela is most useful in the areas where narcotic analgesics are unwarranted and where salicylates are inadequate. Its muscle-relaxant properties are dependable yet significantly free of the limitations or problems often associated with other relaxants.

MYOGESIC: MUSCLE RELAXANT ANALGESIC

Rela relaxes acute muscle spasm
Relief of muscle spasm (excellent to good effectiveness in the majority of patients).[1]

Rela provides persistent pain relief through its relaxant and analgesic actions
"Relief from pain was usually rapid and sometimes dramatic."[1]

Rela provides comfort free of spasm and pain
"A number of patients reported freedom from insomnia which they attributed to freedom from pain."[1]

1. Kuge, T.: To be published.

Schering

H-247

Why Clinical Judgment Often Dictates Altafur for Peroral, Systemic Therapy of Pyodermas

Gratifying Therapeutic Response

ALTAFUR was found "highly satisfactory in most of the primary and secondary bacterial dermatoses treated to date," including "pyodermas . . . caused by antibiotic resistant strains of staphylococci."[1] In a nationwide survey[2] there were **94%** satisfactory results (cured or improved) among 159 patients treated with ALTAFUR for pyodermas.

Virtually Uniform in vitro Susceptibility of Staphylococcus aureus

99.5% of isolates (214 of 215) from patients with staphylococcal infections—including many antibiotic-resistant strains—proved sensitive in vitro to ALTAFUR in tests conducted across the nation.[3] **99.7%** of staphylococcal isolates (334 of 335) at a large general hospital—including many antibiotic-resistant strains—proved sensitive in vitro to ALTAFUR.[4]

Wide, Stable Antimicrobial Spectrum

"Because of its relationship to previously developed nitrofurans, it is anticipated that [ALTAFUR] will retain its original spectrum after longstanding clinical usage."[5] Development of significant bacterial resistance to ALTAFUR has not been encountered to date.[6]

Minimal Side Effects

Side effects are easily avoided or minimized by these simple precautions: 1) *alcohol should not be ingested in any form, medicinal or beverage, during* ALTAFUR *therapy and for one week thereafter* 2) each dose should be taken with or just after meals, and with food or milk at bedtime (to reduce the likelihood of occasional nausea and emesis).

---

1. Weiner, A. L.: Paper presented at the Conference on Recent Advances in the Treatment of Chronic Dermatoses, University of Cincinnati (Ohio), Nov. 5, 1959. 2. Compiled by the Medical Department, Eaton Laboratories, from case histories received. 3. Christenson, P. J., and Tracy, C. H.: Current Therapeutic Research 2:22, 1960. 4. Glas, W. W., and Britt, E. M.: Proceedings of the Detroit Symposium on Antibacterial Therapy, Michigan and Wayne County Academies of General Practice, Detroit, Sept. 12, 1959, p. 14. 5. Leming, B. H., Jr.: Ibid., p. 22. 6. Investigators' reports to the Medical Department, Eaton Laboratories.

Tablets of 250 mg. (adult)

and 50 mg. (pediatric)

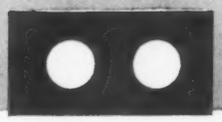
bottles of 20 and 100

Altafur®

brand of furazolium

NITROFURANS . . . a unique class of antimicrobials

EATON LABORATORIES, NORWICH, NEW YORK

Just two tablets  *at bedtime*

RAUWILOID®

alseroxylon, 2 mg.

...does more than lower blood pressure!

Seven years of experience show
that RAUWILOID also affords

Safety based on negligible incidence of side actions

Freedom from concern over sudden hypotensive episodes or unwanted biochemical alterations

Practicality.. simplicity of dosage
.. applicable to a wide range of patients

When more potent drugs are needed, prescribe one of the *convenient single-tablet combinations*

Rauwiloid® + Veriloid®
alseroxylon 1 mg. and alkavervir 3 mg.

or

Rauwiloid® + Hexamethonium
alseroxylon 1 mg. and hexamethonium chloride dihydrate 250 mg.

Many patients with severe hypertension can be maintained on Rauwiloid alone after desired blood pressure levels are reached with combination medication.

Riker

Northridge, California

Cremomycin® provides rapid relief of virtually all diarrheas

NEOMYCIN—rapidly bactericidal against most intestinal pathogens, but relatively ineffective against certain diarrhea-causing organisms.

SULFASUXIDINE® (succinylsulfathiazole)—an ideal adjunct to neomycin because it is highly effective against Clostridia and certain other neomycin-resistant organisms.

KAOLIN AND PECTIN—coat and soothe the inflamed mucosa, adsorb toxins, help reduce intestinal hypermotility, help provide rapid symptomatic relief.

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.

MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

CREMOMYCIN AND SULFASUXIDINE ARE TRADEMARKS OF MERCK & CO., INC.

EVEN HER DOCTOR DOESN'T KNOW . . . (she has dandruff). The great lavender coverup. Too many people take this wig-on-the-head attitude toward dandruff . . . if you can't see it, it isn't there. (Besides which, they've tried all the "sure cures" anyway and yet they're still scratching.) Someone should tell them there's a medical answer to dandruff control —Selsun.® It could be that an authoritative word from you . . . and a prescription for Selsun . . . would be the best thing that could happen to your next scalp-scratcher.

SELSUN®
AN ETHICAL ANSWER TO A MEDICAL PROBLEM
SUSPENSION

©1960-ABBOTT LABORATORIES, NO. CHICAGO, ILL. 005017

SELSUN — Selenium Sulfide, Abbott.

ABBOTT

ANEMIA OF PREGNANCY

another indication for

filmtab® **Iberol®**

potent antianemia therapy plus the complete B-complex

2 IBEROL FILMTABS A DAY SUPPLY:

The Right Amount of Iron

Ferrous Sulfate, U.S.P.	1.05 Gm.
(Elemental Iron—210 mg.)	

Plus the Complete B-Complex

Vitamin B_{12} with Intrinsic Factor Concentrate, 1 U.S.P. Unit (Oral)	
Liver Fraction 2, N.F.	200 mg.
Folic Acid	2 mg.
Thiamine Mononitrate	6 mg.
Riboflavin	6 mg.
Nicotinamide	30 mg.
Pyridoxine Hydrochloride	3 mg.
Calcium Pantothenate	6 mg.

Plus Vitamin C

Ascorbic Acid	150 mg.

® FILMTAB—FILM-SEALED TABLETS, ABBOTT
© 1960—ABBOTT LABORATORIES 005018

now! by mouth! a liquid bronchodilator terminates acute asthma in minutes with virtually no risk of gastric upset

ELIXOPHYLLIN®

oral liquid

Following oral dosage of 75 cc. Elixophyllin, mean blood levels of theophylline at 15 minutes[1] exceed those produced by 300 mg. aminophylline I.V.[2]—and therapeutically effective[3] levels persist for hours.[1]

- No sympathomimetic stimulation
- No barbiturate depression
- No suppression of adrenal function

Each tablespoonful (15 cc.) contains theophylline 80 mg. (equivalent to 100 mg. aminophylline) in a hydroalcoholic vehicle (alcohol 20%).

For acute attacks: Single dose of 75 cc. for adults; 0.5 cc. per lb. of body weight for children.

For 24 hour control: For adults 45 cc. doses before breakfast, at 3 P.M., and before retiring; after two days, 30 cc. doses. Children, 1st 6 doses 0.3 cc.—then 0.2 cc. (per lb. of body weight) as above.

1. Schluger, J. et al.: Am. J. Med. Sci. 233:296, 1957.
2. Bradwell, E. K.: Acta med. scand. 146:123, 1953.
3. Truitt, E. B. et al.: J. Pharm. Exp. Ther. 100:309, 1950.

REFER TO PDR
PAGE 812

Sherman Laboratories

Detroit 11, Michigan

in bacterial infections the new alternative:

℞

Madribon 0.5gm

#16

Sig – Tab ii stat

then tab i

once a day –

The *low cost* antibacterial prescription with assured *safety* and *effectiveness*

MADRIBON

safe · effective · economical

"...its simplicity of administration, safety, clinical response and reasonable cost make... [Madribon] a desirable drug in instances where it is equally effective [as the antibiotics] and a choice drug in many antibiotic-resistant cases."[1]

Clinically effective for infections with cultures positive for:

*Staphylococcus aureus hemolyticus**	*B. proteus*
beta hemolytic streptococci	*E. coli**
pneumococci	Proteus*
K. pneumoniae	Shigella
H. influenzae	Salmonella*
*Ps. aeruginosa**	paracolon bacilli

A new alternative in bacterial infections for many reasons—

- wide-spectrum activity
- high rate of clinical effectiveness—up to 90%
- less than 2 per cent side effects—even in long-term use
- minimal risk of hazardous superinfections
- essentially no danger of anaphylactic reactions
- fewer problems with the development of resistant mutants
- economical therapy
- **reserves antibiotic effectiveness for fulminating, life-threatening infections**

For complete information on dosage forms, dosage schedules and precautions, consult literature available on request.

**Some infections due to antibiotic-resistant strains have responded to Madribon.*

ROCHE **ROCHE** LABORATORIES
Division of Hoffmann-La Roche Inc.
Nutley 10, N. J.

The fastest growing antibacterial bibliography:

1. M. J. Mosely, Jr., *J. Nat. M.A.*, *51*:258, 1959.
2. J. C. Elia, *Antibiotic Med. & Clin. Therapy*, *6*: (Suppl. 1), 61, 1959.
3. J. C. Elia, *Ann. New York Acad. Sc.*, *82*: (Art. 1), 52, 1959.
4. E. H. Townsend, Jr. and A. Borgstedt, *Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 64.
5. Reports on file, Roche Laboratories.
6. B. A. Koechlin, W. Kern and R. Engelberg, *Antibiotic Med. & Clin. Therapy*, *6*: (Suppl. 1), 22, 1959.
7. W. A. Leff, *ibid.*, p. 44.
8. T. D. Michael, *ibid.*, p. 57.
9. H. P. Ironson and C. Patel, *ibid.*, p. 40.
10. W. P. Boger, *Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 48.
11. B. H. Leming, Jr., C. Flanigan, Jr. and B. R. Jennings, *Antibiotic Med. & Clin. Therapy*, *6*: (Suppl. 1), 32, 1959.
12. S. Ross, J. R. Puig and E. A. Zaremba, *Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 56.
13. J. D. Young, Jr., W. S. Kiser and O. C. Beyer, *Antibiotic Med. & Clin. Therapy*, *6*: (Suppl. 1), 53, 1959.
14. J. F. Glenn, J. R. Johnson and J. H. Semans, *ibid.*, p. 49.
15. C. W. Daeschner, *Ann. New York Acad. Sc.*, *82*: (Art. 1) 64, 1959.
16. S. Guss and A. J. Spiro, *Pediatric Conferences*, *2*:14, 1959.
17. R. J. Schnitzer and W. F. DeLorenzo, *Antibiotic Med. & Clin. Therapy*, *6*: (Suppl. 1), 17, 1959.
18. R. J. Schnitzer, W. F. DeLorenzo, E. Grunberg and R. Russomanno, *Proc. Soc. Exper. Biol. & Med.*, *99*:421, 1958.
19. W. F. DeLorenzo and R. Russomanno, *Antibiotic Med. & Clin. Therapy*, *6*: (Suppl. 1), 14, 1959.
20. B. Fust and E. Boehni, *ibid.*, p. 3.
21. W. F. DeLorenzo and A. M. Schumacher, *ibid.*, p. 11.
22. O. Brandman, C. Oyer and R. Engelberg, *J. M. Soc. New Jersey*, *56*:24, 1959.
23. L. O. Randall, R. E. Bagdon and R. Engelberg, *Toxicol. & Appl. Pharmacol.*, *1*:28, 1959.
24. S. M. Finegold, Z. Kudinoff, H. O. Kendall and V. E. Kvinge, *Ann. New York Acad. Sc.*, *82*: (Art. 1), 44, 1959.
25. W. J. Grace, *ibid.*, p. 51.
26. L. E. Skinner, *ibid.*, p. 57.
27. S. W. Levy, *ibid.*, p. 80.
28. M. M. Cahn and E. J. Levy, *ibid.*, p. 84.
29. M. Sierp and J. W. Draper, *ibid.*, p. 92.
30. G. A. Moore, *ibid.*, p. 61.
31. W. P. Boger and J. J. Gavin, *ibid.*, p. 18.
32. W. S. Kiser, O. C. Beyer and J. D. Young, *ibid.*, p. 105.
33. B. H. Leming, Jr. and C. Flanigan, Jr., *ibid.*, p. 31.
34. R. E. Bagdon, L. O. Randall and W. A. Leff, *ibid.*, p. 3.
35. W. F. DeLorenzo and R. J. Schnitzer, *ibid.*, p. 10.
36. G. Carroll, Discussant, *ibid.*, p. 110.
37. S. Krugman, Discussant, *ibid.*, p. 78.
38. E. H. Townsend, Jr. and A. Borgstedt, *ibid.*, p. 71.
39. T. D. Michael, *ibid.*, p. 40.
40. A. E. Thill, *Pennsylvania M. J.*, *62*:1534, 1959.
41. J. C. Elia, *Mil. Med.*, in press.
42. B. Wolach, *Colorado GP*, *1*:4, 1959.
43. H. L. Rosenthal and L. Jud, *J. Lab. & Clin. Med.*, *54*:461, 1959.
44. R. E. Ray, *Case Rep. Child. Mem. Hosp., Chicago*, *17*:4445, 1959.
45. P. Rentchnick and J. Lagier, *Schweiz. med. Wchnschr.*, *89*:894, 1959.
46. J. Leng-Levy, J. David-Chausse, P. Gibaud and J. Bottin, *J. méd. Bordeaux*, *136*: (6), 713, 1959.
47. B. H. Leming, Jr. and C. Flanigan, Jr., Scientific Exhibit, Annual Meeting of the American Medical Association, Atlantic City, N. J., June, 1959.
48. J. C. Elia, *ibid.*
49. O. Thalhammer (University Pediatric Clinic, Vienna, Austria), paper presented at the International Congress of Infectious Pathology, Milan, Italy, May 6-10, 1959.
50. R. Schuppli (Director, University Dermatological Clinic, Basle, Switzerland), *ibid.*
51. S. Rummelhardt (First University Surgical Clinic, Vienna, Austria), *ibid.*
52. M. Rinetti (Institute of Surgical Pathology, University of Parma, Italy), *ibid.*
53. M. Rentsch (University Pediatric Clinic, Berne, Switzerland), *ibid.*
54. N. Quattrin (Cardarelli Hospital, Naples, Italy), *ibid.*
55. E. Picha (First University Gynecological Clinic, Vienna, Austria), *ibid.*
56. R. Neimeier (University Gynecology Clinic, Basle, Switzerland), *ibid.*
57. G. Moustardier (Faculty of Medicine and St. Andrew's Hospital, Bordeaux, France), *ibid.*
58. S. T. Madsen (Bergen, Norway), *ibid.*
59. W. P. Boger, *ibid.*
60. P. Buenger (Medical Department, Heidberg General Hospital, Langenhorn, Hamburg, Germany), *ibid.*
61. H. Ptasnik, *Medizinische*, (31/32), 1437, 1959.

MADRIBON® — 2,4-dimethoxy-6-sulfanilamido-1,3-diazine

in diabetes:

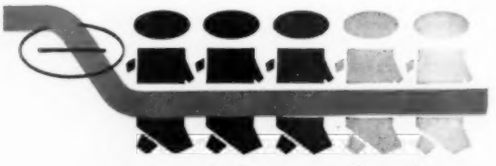
to ensure control where diet alone has failed

to replace or reduce insulin dosage

to realize the full potential of oral therapy

DIABINESE®

brand of chlorpropamide



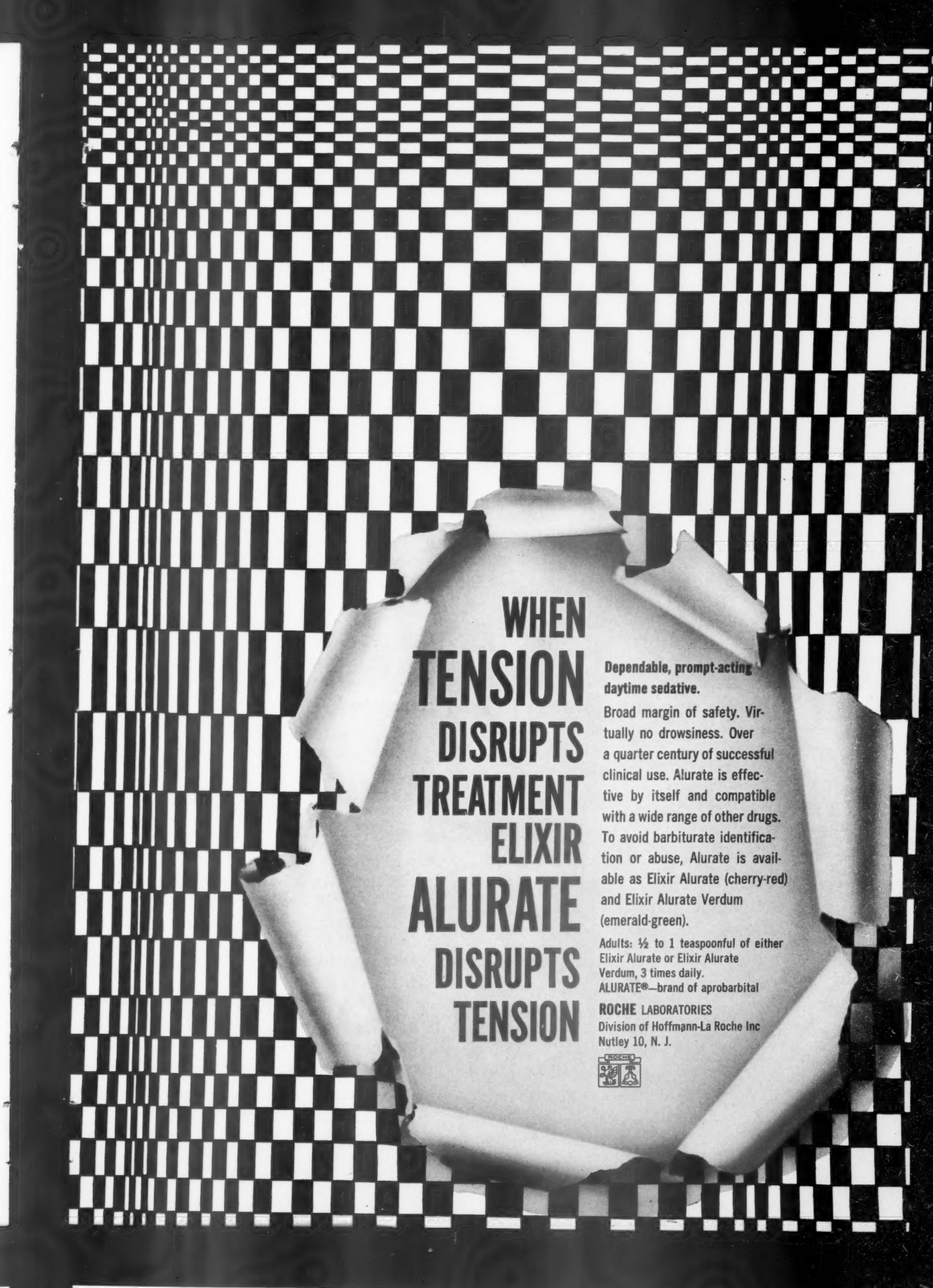
economical once-a-day dosage

available as 100 mg. and 250 mg. scored tablets

Professional information

available to physicians on request.

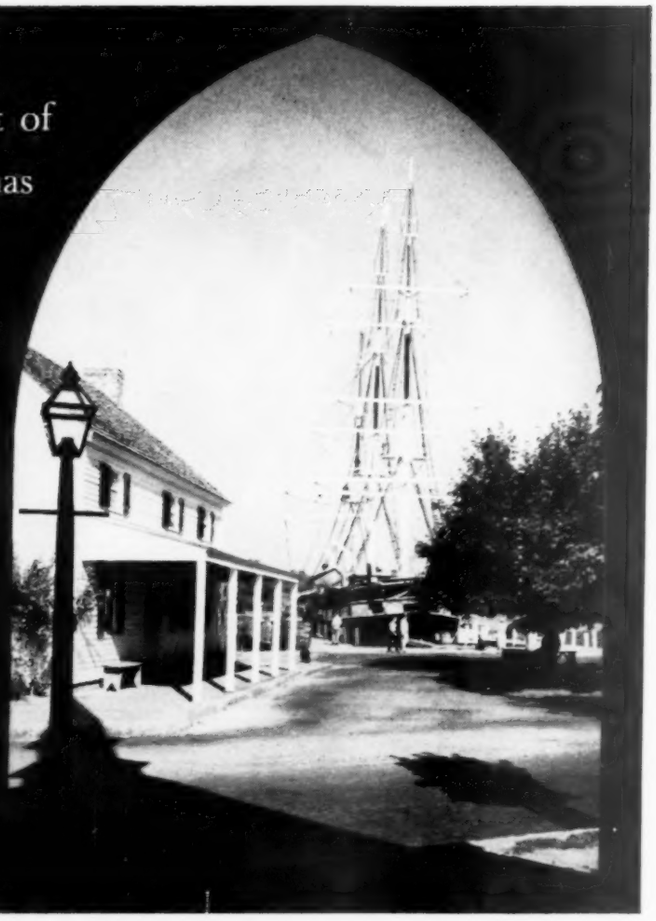
PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. Brooklyn 6, New York Pfizer *Science for the world's well-being™*


WHEN
TENSION
DISRUPTS
TREATMENT
ELIXIR
ALURATE
DISRUPTS
TENSION
Dependable, prompt-acting daytime sedative.
Broad margin of safety. Virtually no drowsiness. Over a quarter century of successful clinical use. Alurate is effective by itself and compatible with a wide range of other drugs. To avoid barbiturate identification or abuse, Alurate is available as Elixir Alurate (cherry-red) and Elixir Alurate Verdum (emerald-green).
Adults: ½ to 1 teaspoonful of either Elixir Alurate or Elixir Alurate Verdum, 3 times daily.
ALURATE®—brand of aprobarbital
ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc
Nutley 10, N. J.
ROCHE

from the New England Journal of Medicine:

"The most striking result of this [Singoserp] study has been the relief of the undesirable side effects produced by other rauwolfia preparations."*

*Bartels, C. C.: New England J. Med. *261*:785 (Oct. 15) 1959.



results you can confirm in your practice:

"In 24 cases syrosingopine was substituted for the rauwolfia product because of 26 troublesome side effects; these symptoms were relieved in all but 3 patients."*

Side Effects	Incidence with Prior Rauwolfia Agent	Incidence with Singoserp
Depression	11	1
Lethargy or fatigue	5	0
Nasal congestion	7	0
Gastrointestinal disturbances	2	2
Conjunctivitis	1	0

(Adapted from Bartels*)

many hypertensive patients prefer

Singoserp®

(syrosingopine CIBA)

because it lowers their blood pressure without rauwolfia side effects

Tablets, 1 mg. (white, scored); bottles of 100.

CIBA
SUMMIT, NEW JERSEY

Complete information available on request.

2/2773MB

Sterosan®-hydrocortisone

brand of chlorquinaldol with hydrocortisone

in the treatment of common dermatoses

effective in more than 80 per cent of cases

Of the reports[1-4] published on Sterosan-hydrocortisone, never has the combined cure and improvement rate been recorded as less than 80 per cent . . . and in two reports[2,4] the rate has exceeded 90 per cent.

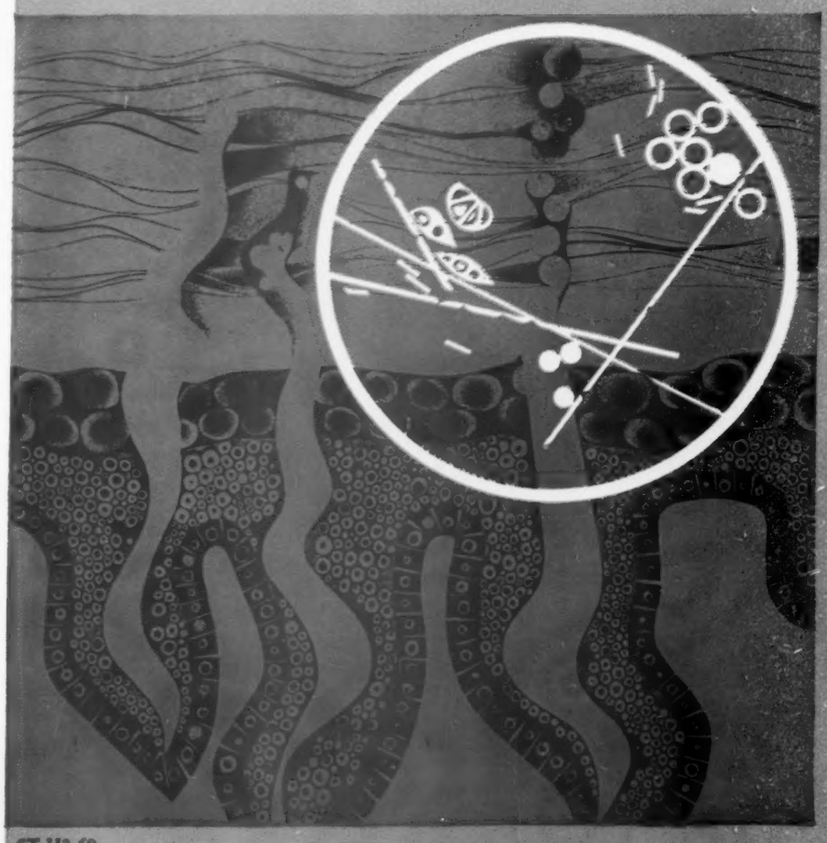
Sterosan-hydrocortisone is effective in virtually all types of common dermatoses and, in particular, in eczematized lesions and those characterized by bacterial or fungous infection.[1]

Available in both cream and ointment forms, Sterosan-hydrocortisone is cosmetically acceptable to the patient and is virtually devoid of sensitizing or irritating properties.

References:
1. Fox, H. H.: Antibiotic Med. & Clin. Therapy *6*:85, 1959. 2. Lubowe, I. I.: Antibiotic Med. & Clin. Therapy *4*:81, 1957. 3. Murphy, J. C.: Rocky Mountain M. J. 55:53 (June), 1958. 4. Pace, B. F.: M. Rec. & Ann. *51*:370, 1957.

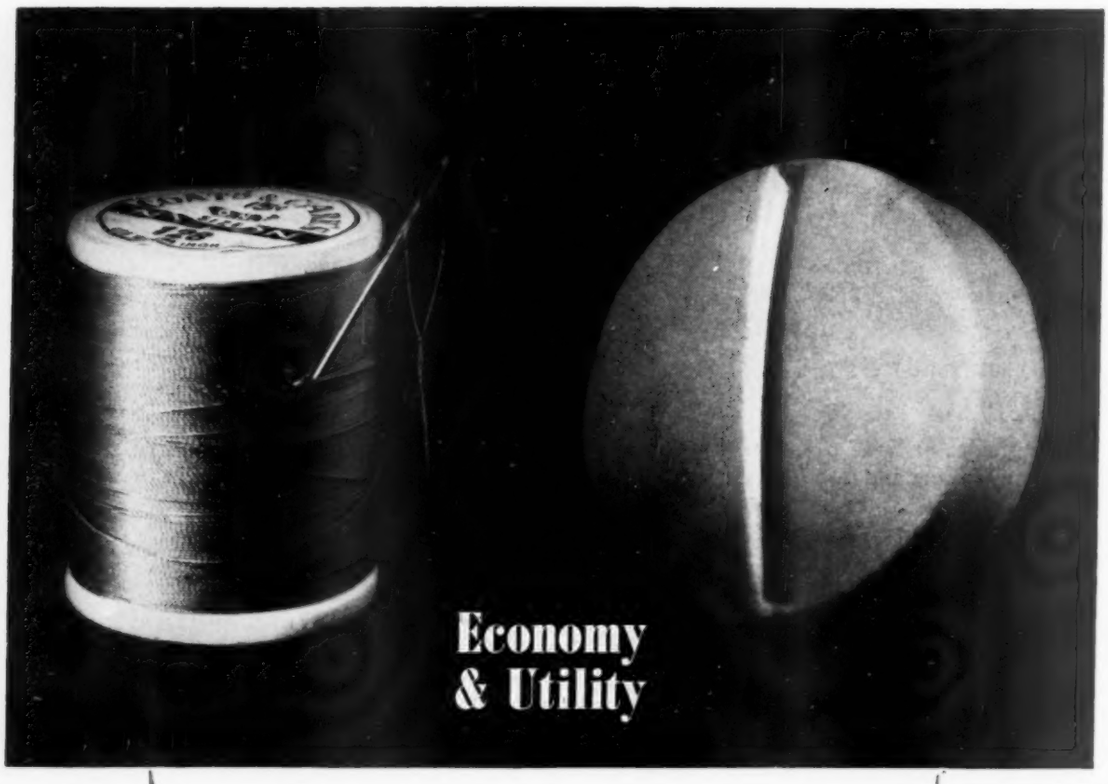
Sterosan®-hydrocortisone, brand of chlorquinaldol with hydrocortisone, is available as Cream or Ointment each containing 3% of chlorquinaldol and 1% of hydrocortisone. In tubes of 5 Gm. and 20 Gm. Prescription only.

Geigy, Ardsley, New York



Geigy

ST-H2-60



clinically proved oral penicillin therapy that costs your patients less

Pentids

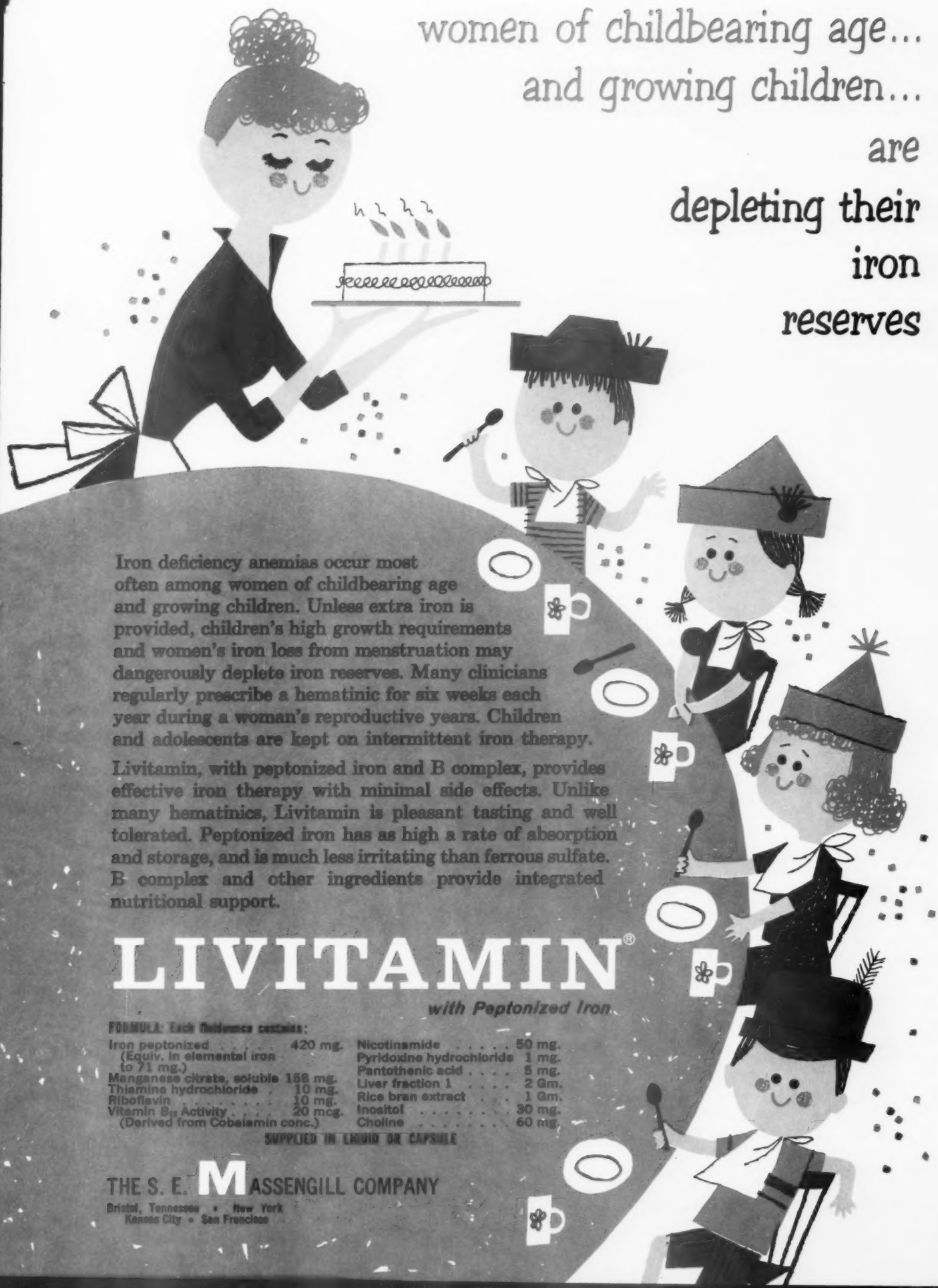
Squibb Penicillin G Potassium

Available in these convenient dosage forms: PENTIDS '400' TABLETS (400,000 u.) • PENTIDS '400' FOR SYRUP (400,000 u. per 5 cc. when prepared) • PENTIDS TABLETS (200,000 u.) • PENTIDS FOR SYRUP (200,000 u. per 5 cc. when prepared) • PENTID-SULFAS TABLETS (200,000 u. with 0.5 Gm. triple sulfas) • PENTIDS CAPSULES (200,000 u.) • PENTIDS SOLUBLE TABLETS (200,000 u.)

"PENTIDS"® IS A SQUIBB TRADEMARK

SQUIBB

Squibb Quality—the Priceless Ingredient



women of childbearing age...
and growing children...
are
depleting their
iron
reserves

Iron deficiency anemias occur most often among women of childbearing age and growing children. Unless extra iron is provided, children's high growth requirements and women's iron loss from menstruation may dangerously deplete iron reserves. Many clinicians regularly prescribe a hematinic for six weeks each year during a woman's reproductive years. Children and adolescents are kept on intermittent iron therapy.

Livitamin, with peptonized iron and B complex, provides effective iron therapy with minimal side effects. Unlike many hematinics, Livitamin is pleasant tasting and well tolerated. Peptonized iron has as high a rate of absorption and storage, and is much less irritating than ferrous sulfate. B complex and other ingredients provide integrated nutritional support.

LIVITAMIN®

with Peptonized Iron

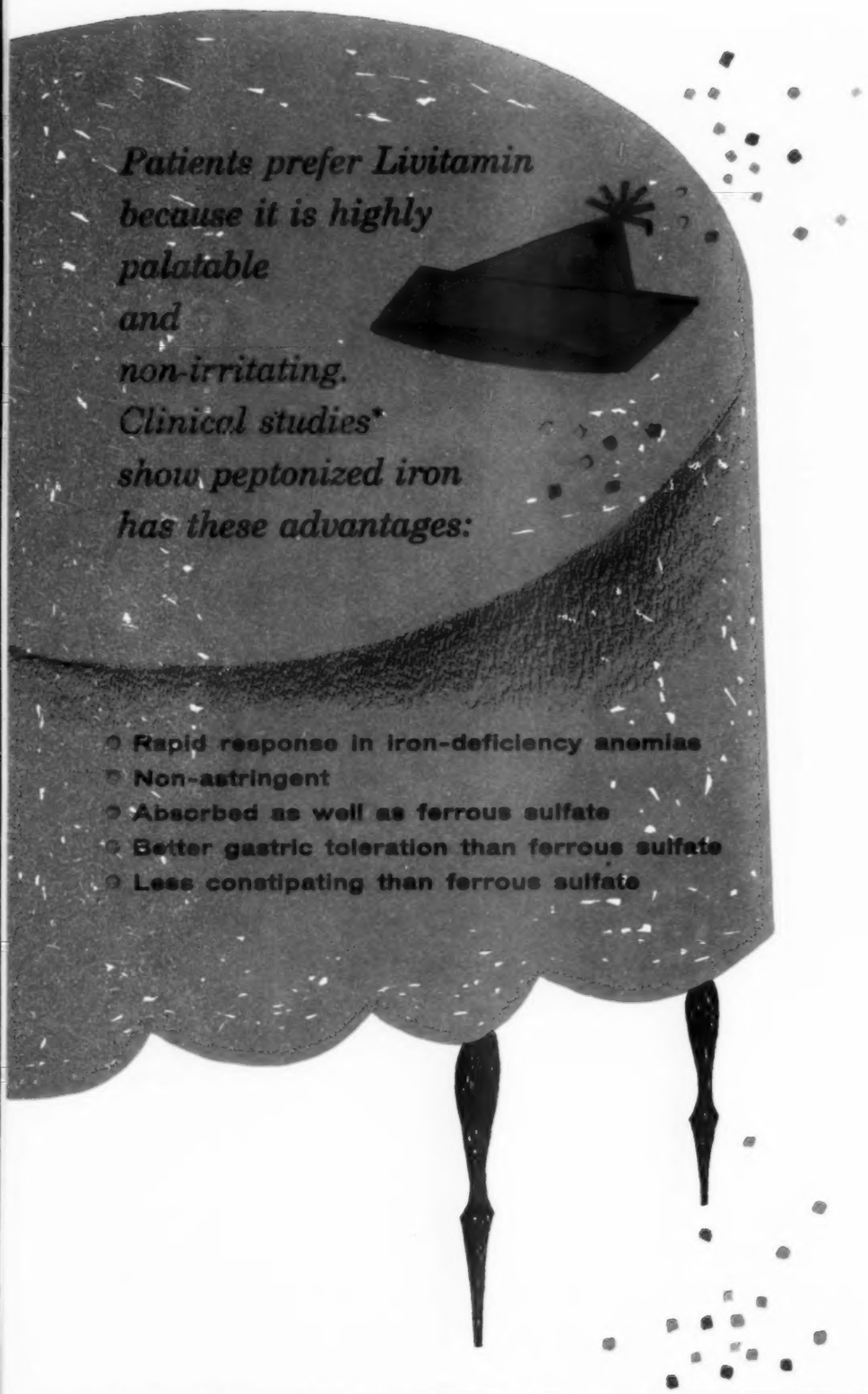
FORMULA: Each fluidounce contains:

Iron peptonized (Equiv. to 71 mg. elemental iron)	420 mg.	Nicotinamide	50 mg.
Manganese citrate, soluble	158 mg.	Pyridoxine hydrochloride	1 mg.
Thiamine hydrochloride	10 mg.	Pantothenic acid	5 mg.
Riboflavin	10 mg.	Liver fraction 1	2 Gm.
Vitamin B_{12} Activity (Derived from Cobalamin conc.)	20 mcg.	Rice bran extract	1 Gm.
		Inositol	30 mg.
		Choline	60 mg.

SUPPLIED IN LIQUID OR CAPSULE

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee • New York
Kansas City • San Francisco

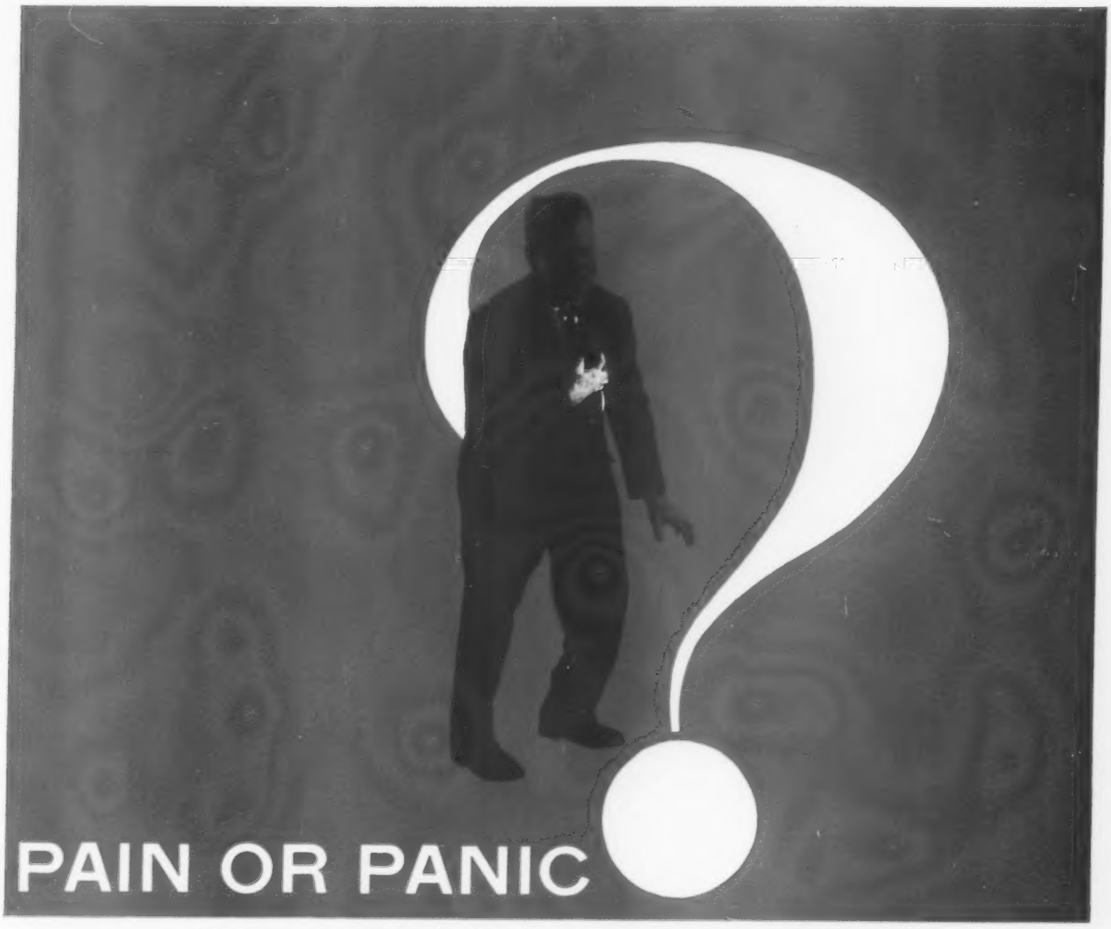


LIVITAMIN® *with Peptonized Iron*

. . . the preferred hematinic

*Keith, J.H.: Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate, Am. J. Clin. Nutrition 1:35 (Jan.-Feb., 1957).

THE S. E. MASSENGILL COMPANY Bristol, Tennessee • New York • Kansas City • San Francisco



Your angina patient hardly knows where one leaves off and the other begins. What he does recognize is the relief he gets from **both** when you prescribe CARTRAX.

FOR ANGINAL PAIN

PETN (pentaerythritol tetranitrate) is "... the most effective drug currently available for prolonged prophylactic treatment of angina pectoris."[1]

FOR CARDIAC PANIC

"I favor ATARAX [as the tranquilizer for the anxious cardiac] ... because there is an absence of side effects with this drug, and also because in cardiacs who are troubled with ectopic beats, ATARAX has a quinidine-like action."[2]

PETN + ATARAX®*

TOGETHER ONLY IN

CARTRAX®

Dosage: Begin with 1 to 2 yellow CARTRAX "10" tablets (10 mg. PETN plus 10 mg. ATARAX) 3 to 4 times daily. When indicated, this may be increased by switching to pink CARTRAX "20" tablets (20 mg. PETN plus 10 mg. ATARAX). For convenience, write "CARTRAX 10" or "CARTRAX 20." **Supplied:** In bottles of 100. Prescription only.

References: 1. Russek, H. I.: Postgrad. Med. 19:562 (June) 1956. 2. Russek, H. I.: Presented at the Symposium on the Management of Cardiovascular Problems of the Aged, Dade County Medical Association, Miami Beach, April 12, 1958.

*brand of hydroxyzine

ROERIG Rx

New York 17, N. Y.
Division, Chas. Pfizer & Co., Inc.
Science for the World's Well-Being™

from carcinomatosis

Tigan

Even in the most severe emetic attacks to which cancer patients are subject, Tigan provides effective control—therapeutic or prophylactic. To patients undergoing RADIATION for multiple metastases and metastatic lesions, "...Tigan was truly a godsend, enabling them to continue treatment and obtain relief...not obtainable in any other non-surgical way."[1] Of 63 patients in terminal stages of cancer, 57 were completely or partially relieved of nausea/vomiting due to radiation.[2] Where CHEMOTHERAPEUTIC AGENTS had previously caused both nausea and vomiting, Tigan permitted large doses of therapy to be given "...without the associated nausea and vomiting that we had seen before."[3] And in nausea and vomiting due to UNDERLYING NEOPLASTIC DISEASE, Tigan produced a good-to-excellent response in 58 per cent and a fair response in 21 per cent.[3,4]

In addition to the effectiveness required in challenging conditions, Tigan provides the safety which assumes special significance in everyday situations. Tigan shares the superior antiemetic potency (and mechanisms of action[5]) of the phenothiazines without entailing any of their risks or, for that matter, any of the side effects

to car sickness

safely controls the entire range of emetic situations without antihistamine side effects and without phenothiazine risks

of the antihistamines.[6-9] No sedation or other side effects were observed in TRAVEL SICKNESS patients, who were quickly and efficiently relieved by Tigan.[10] Nor was any drowsiness or other untoward reaction seen in 16 PREGNANT women, 15 of whom became asymptomatic on Tigan, while other antiemetic medication had failed or caused drowsiness in several.[10] In GASTROINTESTINAL vomiting, where results were "good" in 86 per cent and "fair" in 14 per cent, Tigan was "well tolerated" by all.[11]

TIGAN (COMPARED TO PLACEBO) IN SEASICKNESS

Medication	Number of Patients	RESULTS	
		Improved	Unimproved
Tigan, given initially	142	133	9
Placebo, given initially	74	7	67
Tigan, given after failure of placebo	65	61	4

NO SPECIAL PRECAUTIONS OR CONTRAINDICATIONS—Patients on Tigan may drive an automobile or operate other mechanical equipment without the hazard of drowsiness; and carry on household activities without lethargy or sleepiness.

Available: TIGAN CAPSULES—100 mg, blue and white; TIGAN AMPULS—2 cc (100 mg/cc); TIGAN SUPPOSITORIES—200 mg.

For dosage instructions, consult product literature available on request.

References: 1. J. A. Lucinian and R. H. Bohn, paper read at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959. 2. O. W. Doyle, *Clin. Med.*, 7:43, 1960. 3. B. I. Shnider and L. Gold, paper read at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959. 4. D. W. Molander, *ibid.* 5. W. Schallek, G. A. Heise, E. F. Keith and R. E. Bagdon, *J. Pharmacol. & Exper. Therap.*, *126*:270, 1959. 6. Reports on file, Roche Laboratories. 7. O. C. Brandman, paper read at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959. 8. I. Roseff, W. B. Abrams, J. Kaufman, L. Goldman and A. Bernstein, *J. Newark Beth Israel Hosp.*, *9*:189, 1958. 9. W. B. Abrams, I. Roseff, J. Kaufman, L. Goldman and A. Bernstein, *New York J. Med.*, *59*:4217, 1959. 10. Personal communications. 11. L. McLaughlin, paper read at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959.

TIGAN® Hydrochloride—4-(2-dimethylaminoethoxy)-N-(3, 4, 5-trimethoxybenzoyl) benzylamine hydrochloride

ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc.
Nutley 10, N.J.

WEIGHT GAIN...

WHY DECADRON TREATS THE WHOLE PATIENT MORE EFFECTIVELY

"...WEIGHT GAIN IS A DEFINITE THERAPEUTIC ADVANTAGE..."[1]

Many patients with chronic rheumatoid arthritis or other collagen or allergic diseases require the "tonic effect"[2] as well as the anti-inflammatory effects of dexamethasone. For those, DECADRON has relieved fatigue and weakness,[3,4] stimulated appetite[3-5] and often promoted a "real gain in weight"[5] —frequently a clinical advantage which contributes to optimum therapeutic results.

"SUPERIOR TO OTHER STEROIDS IN CURRENT USE"[2]

The clinical superiority of dexamethasone rests on a unique balance of potency and safety, combining "the most potent"[6] anti-inflammatory action with "the least number of side effects."[7] This superiority has already been confirmed in over 16 million patient-days of treatment with DECADRON.

References: 1. Duvenci, J., et al.: Ann. Allergy *17*:695, 1959. 2. Rudolph, J. A., and Rudolph, B. M.: Ann. Allergy *17*:710, 1959. 3. Spies, T. D., et al.: South. M. J. *51*:1066, 1958. 4. Galli, T., and Mannetti, C.: Minerva med. *50*:949, 1959. 5. Segal, M. S., et al.: Ann. Allergy *17*:413, 1959. 6. Bunim, J. J., et al.: Arthritis & Rheumatism *1*:313, 1958. 7. Silverman, H. I., and Urdang, A.: Am. Prof. Pharm. *25*:531, 1959.

Supplied: As 0.75 mg. and 0.5 mg. scored, pentagon-shaped tablets in bottles of 100 and 1000. Also available as Injection DECADRON Phosphate.

Additional information on DECADRON is available to physicians on request.

DECADRON is a trademark of Merck & Co., Inc.

Decadron®
DEXAMETHASONE

MERCK SHARP & DOHME
Division of Merck & Co., INC., West Point, Pa.

chemical name:
1-m-aminophenyl-2-pyridone
generic name:
amphenidone

with selective action on the central nervous system at both the cerebral and cord levels.

for the treatment of anxiety & tension without causing drowsiness

Dornwal is regarded as a tranquilizer best suited for ambulatory patients.

- does not produce depression or depersonalization
- relieves acute emotional upsets
- relieves tension without undue stimulation
- effectively interrupts tension headaches
- is virtually devoid of sedative activity

Dornwal has proved to be relatively free from side effects when administered at recommended dosage. In 593 patients the incidence of drowsiness was less than 2 per cent — statistically not significant.

Prescribe Dornwal for your next patient who needs a tranquilizer but cannot afford to be drowsy. Write for your trial supply.

Indications: anxiety and tension, various types of psychoneuroses, menopausal syndrome, tension headache, alcoholism, premenstrual tension, behavior problems in children.

Dosage: One or two 200 mg. tablets three times a day. Children, one or two 100 mg. tablets two times a day. Administration limited to three months duration.

Supplied: 200 mg. yellow scored tablets, and 100 mg. pink tablets, each in bottles of 100 and 500.

Maltbie

Maltbie Laboratories Division
Wallace & Tiernan Incorporated
Belleville 9, New Jersey

PDL-03

***orally* effective progestational therapy**

NORLUTIN®

(norethindrone, Parke-Davis)

in conditions involving deficiency of progesterone...

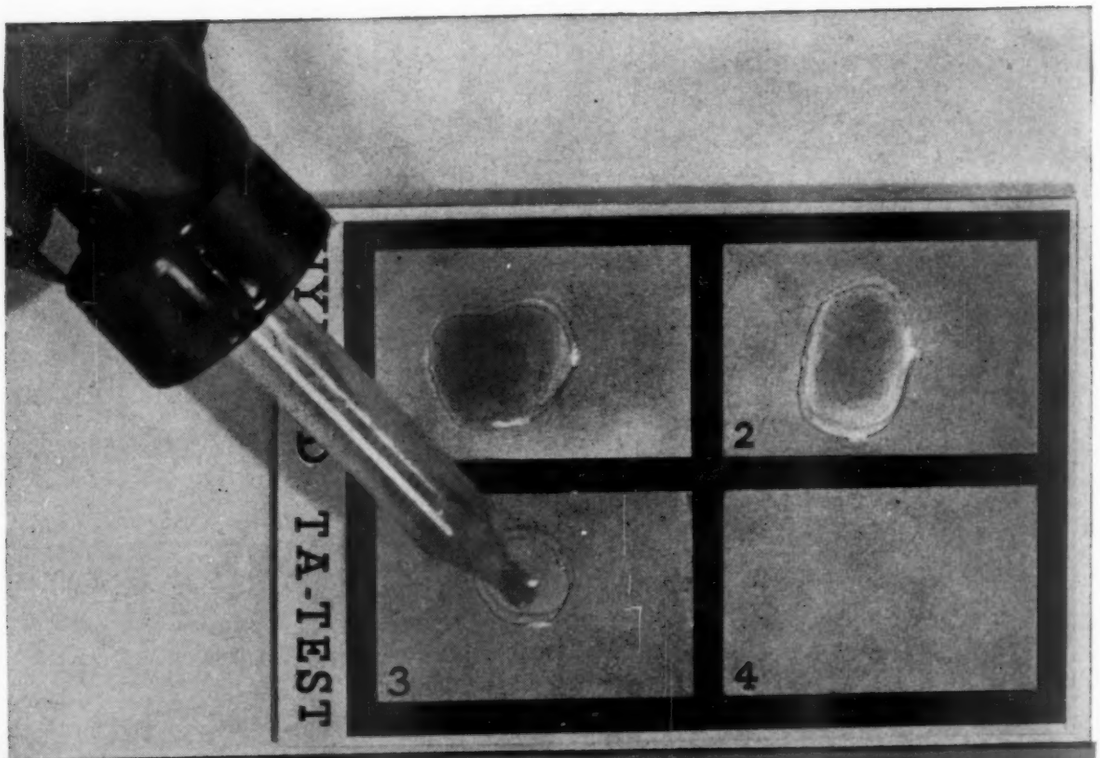
primary and secondary amenorrhea • menstrual irregularity • functional uterine bleeding • endocrine infertility • habitual abortion • threatened abortion • premenstrual tension • dysmenorrhea

PACKAGING: 5-mg. scored tablets, bottles of 30.

12660

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

PARKE-DAVIS



TA-TEST provides an important new time-saving procedure in diagnosis of thyroid disease. It speedily detects thyroglobulin autoprecipitin, associated with Hashimoto's Disease (chronic lymphoid thyroiditis) and primary myxedema. Since both of these conditions are treated medically, rather than surgically, the diagnostic helpfulness of this new screening test is obvious. The test is simple to perform and requires only two drops of patient's serum. Results are then read within two to three minutes. TA-TEST is supplied in compact 20-test kits containing reagent, diluent, control serum and glass slide. List No. 90-100.

Hyland

TA-TEST*

RAPID SLIDE TEST FOR DETECTION OF
THYROGLOBULIN AUTOPRECIPITIN
ASSOCIATED WITH
HASHIMOTO'S DISEASE

HYLAND LABORATORIES
4501 Colorado Blvd.
Los Angeles 39, Calif.
160 Lockwood Ave., Yonkers, N.Y.

*Trademark of Hyland Laboratories

1. Negative specimen, undiluted 2. Negative specimen, diluted
3. Positive control

Schering

SCHERING writes a new chapter in diuretic & hypertension therapy

lowest dosage – unexcelled diuretic activity

Naqua™

trichlormethiazide

selective electrolyte screening

lower potassium excretion, less risk of digitalis toxicity...maximum sodium output... balanced sodium and chloride excretion...24-hour effect on one 4 mg. dose...significant antihypertensive effect alone, potentiates other antihypertensive drugs...
more economically priced...dosage less than 1/100 of chlorothiazide
Packaging: NAQUA Tablets, 2 and 4 mg. scored, bottles of 100 and 1000.

5-579

Photos Courtesy F. C. Gindhart, M.D.

For more successful pregnancies in habitual aborters

When added to your individualized anti-abortive regimen, NUGESTORAL may help you bring more habitual aborters to successful term.

By supplying five therapeutic agents known to contribute to fetal salvage, NUGESTORAL creates an optimal maternal environment for the maintenance of pregnancy.

Nugestoral supplies in each daily dose of three tablets:

Progestoral® (Ethisterone) ..... 45.0 mg.
- Progestational action helps maintain fetus
- Relieves uterine spasticity

Ascorbic Acid (Vitamin C) ..... 525.0 mg.

Purified Hesperidin ..... 487.5 mg.
(equiv. 600 mg. hesperidin complex)
- Prevent or correct abnormal capillary fragility
- Protect and strengthen decidual vessels

Menadione Sodium Bisulfite ..... 6.0 mg.
(U.S.P. Equivalency)
- Prevents hypoprothrombinemia in mother and child

dl, Alpha-Tocopherol Acetate (Vitamin E) ..... 10.5 mg.
- Extra nutritional insurance

DOSAGE: *Prophylactic* – One NUGESTORAL tablet t.i.d. from diagnosis through at least the second trimester.

Symptomatic – Two tablets t.i.d. or q.i.d. until symptoms are controlled. Then one tablet t.i.d.

Available in boxes of 30 and 100. Write for copies of recent clinical reports.

Organon

ORGANON INC., ORANGE, N. J.

Nugestoral®

The
"practical approach"
to
clinical cardiology
is found in

THE AMERICAN JOURNAL
OF CARDIOLOGY

Issued monthly

as the official publication of
The American College
of Cardiology

WRITE FOR YOUR FREE
SAMPLE COPY

The American Journal of Cardiology

11 EAST 36TH STREET
NEW YORK 16, N.Y.

Schering

Naqua™
trichlormethiazide

lowest dosage, unexcelled
activity for control of

**hyper-
tension**

S-503

in peptic ulcer...
both are basic

ALUDROX provides rapid, extended relief of pain in peptic ulcer. Its physiological neutralization is in the range of pH 3 to 5—pepsin is inactivated.

The time-proved combination (4:1) of reactive alumina gel and milk of magnesia in ALUDROX relieves pain, accomplishes healing with no fear of gastrin stimulation, induced constipation or complication of existing constipation.

Wyeth Laboratories Philadelphia 1, Pa.

Tablets Suspension

ALUDROX®

Aluminum Hydroxide with Magnesium Hydroxide, Wyeth

Also available: ALUDROX® SA (Aluminum Hydroxide with Magnesium Hydroxide, Ambutonium Bromide and Butabarbital, Wyeth); Suspension and Tablets.

100 ANNIVERSARY Wyeth®

A Century of Service to Medicine

"Every illness includes some nutritional, metabolic disturbance either directly or indirectly."

Keep informed of new developments in:

Nutrition
Metabolism
Endocrinology
Diet Therapy

Read——
The American Journal of Clinical Nutrition
(issued bimonthly)

WRITE FOR YOUR FREE SAMPLE COPY

The American Journal of Clinical Nutrition

11 EAST 36TH STREET
NEW YORK 16, N.Y.

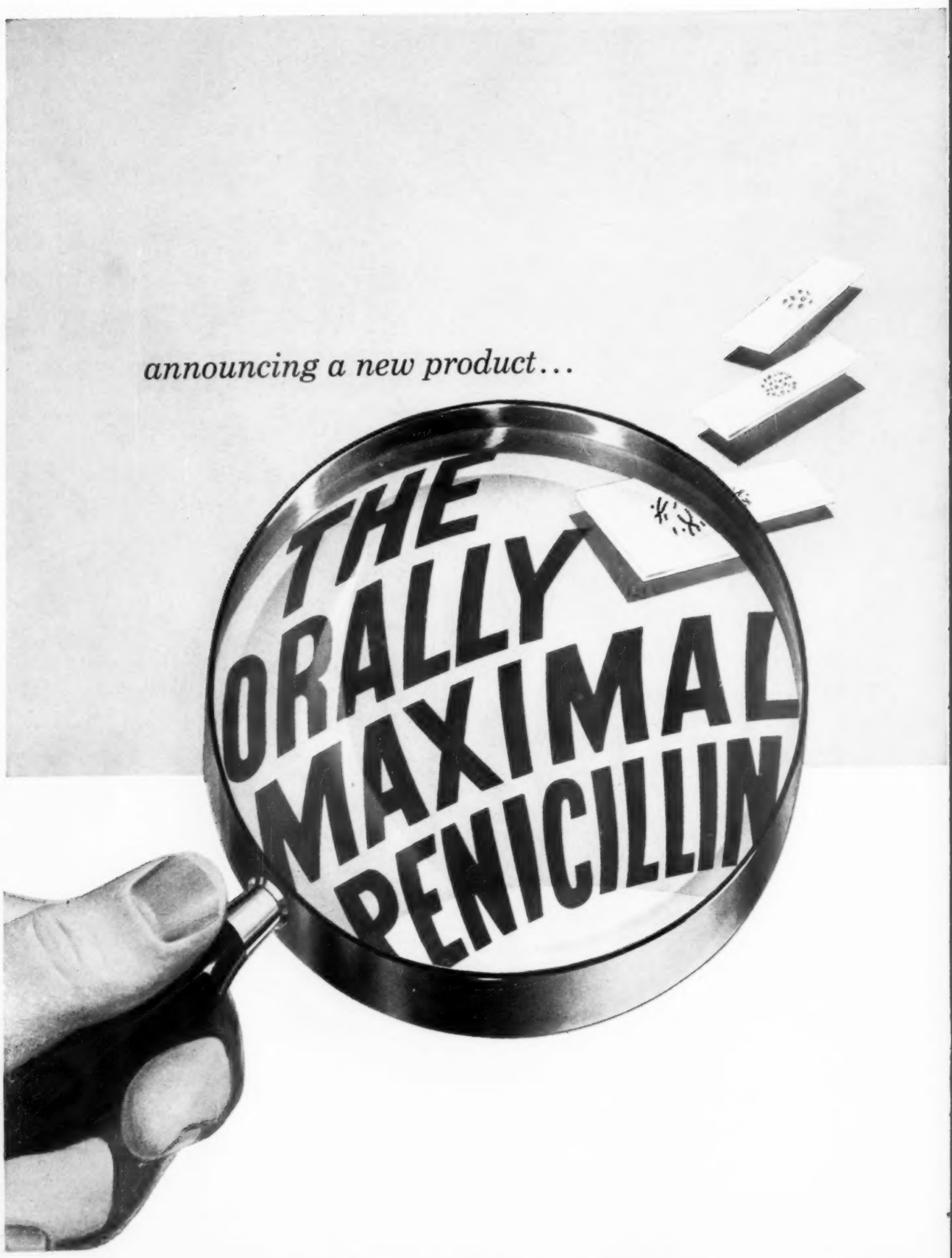
Schering

Naqua™
trichlormethiazide

lowers potassium excretion, minimizes risk of digitalis toxicity in

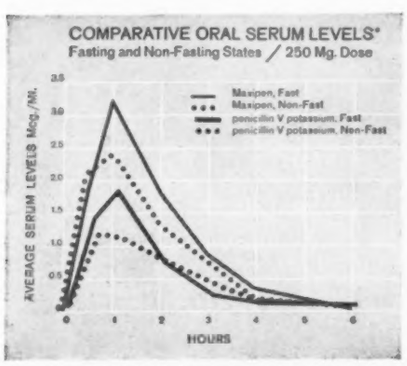
congestive heart failure

5-504


announcing a new product...
THE
ORALLY
MAXIMAL
PENICILLIN

MAXIMAL ABSORPTION Acid stable, extremely soluble. MAXIPEN is rapidly absorbed from the gastrointestinal tract.

MAXIMAL BLOOD LEVELS Substantially higher than potassium penicillin V (higher levels than with intramuscular procaine penicillin G). You get injection levels with a tablet.



*Based on 3294 individual serum antibiotic determinations. Complete details on request.

MAXIMAL FLEXIBILITY May be administered without regard to meals. However, highest absorption is achieved when taken just before or between meals.

MAXIMAL ORAL INDICATIONS Indicated in infections caused by streptococci, pneumococci, susceptible staphylococci, and gonococci, including:

pneumococcal pneumonia	impetigo
gonorrhea	susceptible staphylococcal abscesses (with indicated surgery)
tonsillitis	
laryngitis	
otitis media	cellulitis
streptococcal pharyngitis	lymphangitis
	pyoderma

Also prophylactically in secondary infections following tonsillectomy, dental extractions, other surgical procedures.

Dosage: For moderately severe conditions, 125 to 250 mg. three times daily. For more severe conditions, 500 mg. as often as every four hours around the clock.

Note: To date, MAXIPEN has not shown less allergic reactions than older oral penicillins. Usual precautions regarding administration should be observed.

Supplied: MAXIPEN TABLETS, scored, 125 mg. (200,000 units) bottles of 36; 250 mg. (400,000 units) bottles of 24 and 100.
MAXIPEN FOR ORAL SOLUTION; reconstituted each 5 cc. contains 125 mg., in 60 cc. bottles.

Triumph of Man Over Molecule
Designed by Pfizer for Maximal Benefit

ROERIG Rx

New York 17, N.Y.
J. B. Roerig and Company
Division, Chas. Pfizer & Co., Inc.
Science for the World's Well-Being™

FOR PROVEN MENOPAUSAL BENEFITS with extra relief from anxiety and tension

The vast majority of menopausal women, *especially on the first visit,* are nervous, apprehensive, and tense. PMB-200 or PMB-400 gives your patient the advantage of *extra* relief from anxiety and tension, particularly when the patient is "high strung," under prolonged emotional stress, or when psychogenic manifestations are acute.

Proven menopausal benefits are confirmed by the wide clinical acceptance of "Premarin," specifically for the relief of hot flushes and other symptoms of estrogen deficiency, together with the well established tranquilizing efficacy of meprobamate.

Two potencies to meet the needs of your patients:

PMB 200

"PREMARIN"® WITH MEPROBAMATE*

PMB-200—Each tablet contains conjugated estrogens equine ("Premarin") 0.4 mg., and 200 mg. of meprobamate. When greater tranquilization is necessary you can prescribe PMB-400 – Each tablet contains conjugated estrogens equine ("Premarin") 0.4 mg., and 400 mg. of meprobamate. Both potencies are available in bottles of 60 and 500.

AYERST LABORATORIES
New York 16, N.Y., Montreal, Canada

*MEPROBAMATE, LICENSED UNDER U. S. PAT. NO. 2,724,720. 6917

the margarine clinically proved to lower elevated cholesterol levels

a delicious spread a superb shortening

EMDEE™ margarine

EMDEE™ margarine

substituted for ordinary spreads and solid shortenings helps lower elevated cholesterol levels

Recent investigations demonstrate how effectively cholesterol levels can be significantly reduced by a modified diet which includes the substitution of Emdee Margarine for saturated or hydrogenated spreads and shortenings ordinarily used in the diet.

Eighty per cent of Emdee Margarine's fat content is pure corn oil, whose natural content of polyunsaturated fatty acids has not been destroyed by hydrogenation.* Approximately 42% of its fat content is linoleic acid, an important substance in the control of blood cholesterol levels.

When a patient's intake of the undesirable fats should be reduced, he and his family will welcome Emdee Margarine. It restores natural flavor to a fat-restricted diet and eliminates the chore of preparing special dishes for one member of the family.

On bread, toast and crackers Emdee Margarine has the same taste as other fine spreads, and a firm, smooth texture. It brings back the familiar flavor to baked potatoes, vegetables and popcorn. It can be used for braising, baking, roasting and sautéing, and in white sauces and frostings. It has won praise from Home Economics experts, who found that Emdee Margarine is a high-quality shortening.

Packaged in one-pound cans to protect its fresh taste and firm texture, Emdee Margarine is available only in pharmacies.

PM PITMAN-MOORE COMPANY, DIVISION OF ALLIED LABORATORIES, INC., INDIANAPOLIS 6, INDIANA

REFERENCES: 1. Terman, L. A.: Dietary management of hypercholesterolemia, Geriatrics *14*:111 (Feb.) 1959. 2. Boyer, P. A.; Lowe, J. G.; Gardler, R. W., and Ralston, J. D.: Effect of a practical dietary regimen on serum cholesterol level, J.A.M.A. *170*:257 (May 16) 1959. 3. Vail, Gladys E.: Cooking with fats high in polyunsaturated fatty acids, J. Am. Dietet. A. *35*:119 (Feb.) 1959. 4. Jolliffe, N.; Rinzler, S. H., and Archer, M.: The Anti-Coronary Club; including a discussion of the effects of a prudent diet on the serum cholesterol level of middle-aged men, Am. J. Clin. Nutrition *7*:451 (July-Aug.) 1959.

Reprints of these articles on Emdee Margarine are available on request.

*Special process protected by U. S. Patent No. 2,890,959.

"RISTOCETIN IS AN EFFECTIVE PRIMARY AGENT IN STAPHYLOCOCCAL INFECTIONS"

SPONTIN®

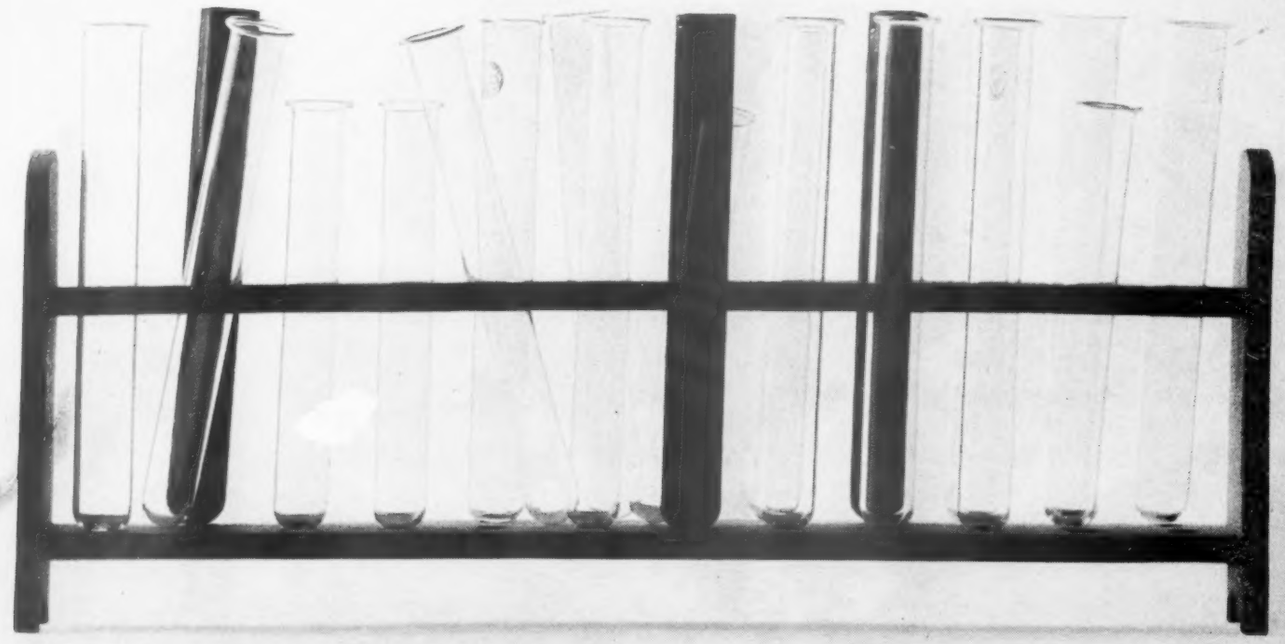
(Ristocetin, Abbott)

CONCLUSIONS—"Ristocetin is an effective primary agent in staphylococcal infections, as well as in short-term therapy of enterococcal endocarditis. It is administered intravenously; intermittent, rapid infusion is recommended. Ristocetin is bactericidal in concentrations attained by this technique . . .

The hematological and other side effects such as phlebitis, skin eruptions, and fever are infrequent with the recommended dosage schedules and mode of administration. The dosage of ristocetin is reduced in renal insufficiency since the antibiotic tends to accumulate.[1]"

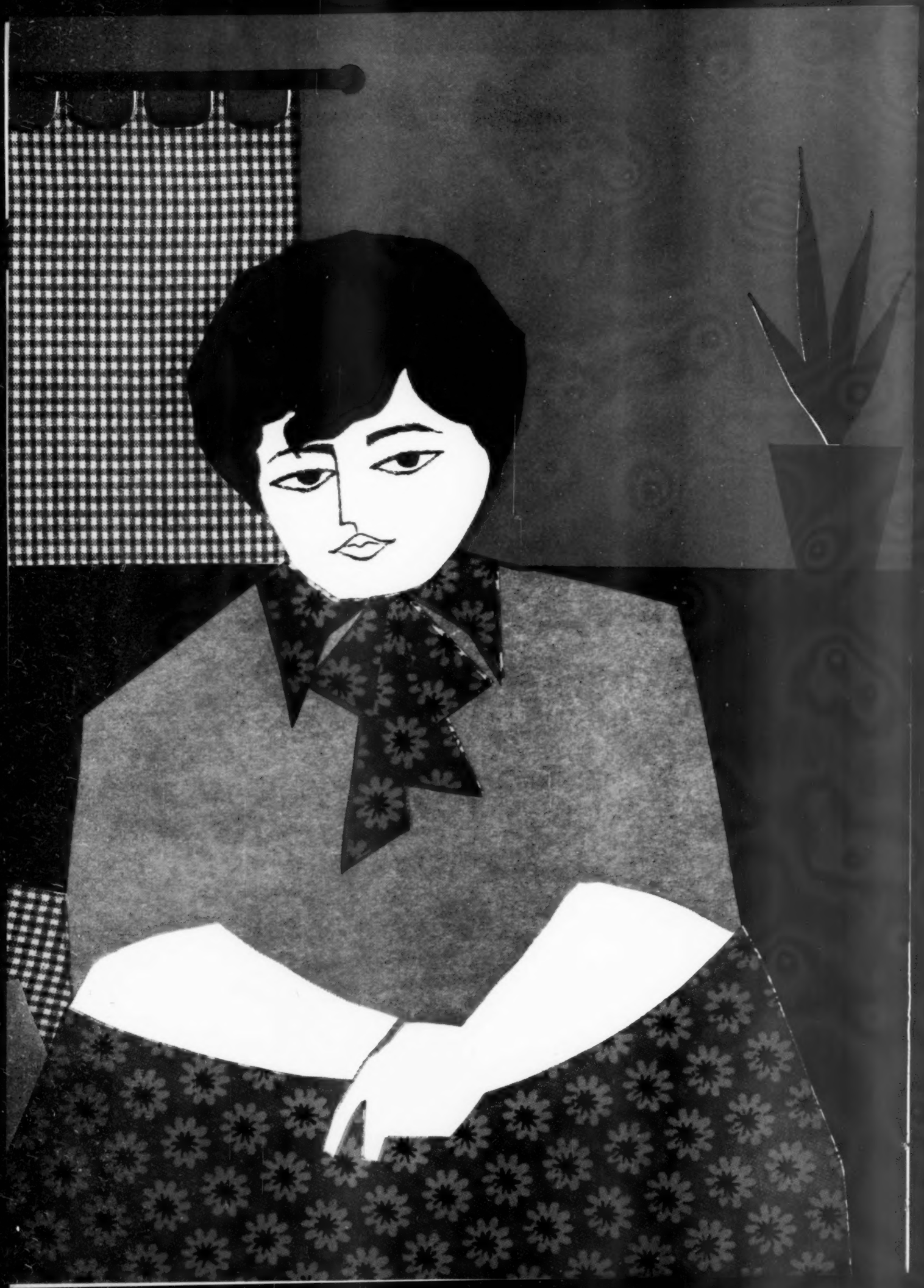
ABBOTT

INDICATIONS: Against staph-, strep-, pneumo- and enterococcal infections. A drug of choice for serious infections caused by organisms that resist other antibiotics. **DOSAGE:** Administered intravenously. A dosage of 25 mg./Kg. daily will usually be adequate for strep-, pneumo- and enterococcal infections. Most staphylococcal infections will be controlled by 25 to 50 mg./Kg. daily. **SUPPLIED:** In vials containing a sterile, lyophilized powder, representing 500 mg. of ristocetin A activity.



1. Romansky, M. J., Ristocetin, Antibiotics Monographs, No. 12, New York, Medical Encyclopedia Inc., 1959.

002193



measured calories for adequate nutrition with high satiety on 900 calories a day—without appetite depressants

METRECAL™

DIETARY FOR WEIGHT CONTROL

adequate nutrition in 900-calorie program

One-half pound of Metrecal powder, the daily 900-calorie feeding, provides 70 grams of high-grade protein plus vitamins and minerals to meet or exceed all established minimum daily requirements. Metrecal itself *is* the diet—the *complete* diet.

impressive clinical results

in uncomplicated overweight: In a twelve-day study of 100 patients[1] on the Metrecal 900-calorie program, the average total weight loss per subject was 6½ pounds (over ½ pound a day). In another study,[2] patients on the 900-calorie Metrecal program for two to thirty weeks showed a weight loss per patient of 3 to 5 pounds weekly during the initial weeks and 2 to 2½ pounds per patient per week thereafter.

in overweight complicated by illness: In a study of 42 overweight patients,[3] some with serious medical disorders such as arthritis, cardiovascular disease, diabetes mellitus and gout, the 900-calorie daily Metrecal program provided an average total weight loss of 6.3 pounds per patient during the first week. The investigator encountered no significant complications in the management of these patients.

excellent patient cooperation

All investigators[1,2,3] commented on excellent patient cooperation. This was attributed to the effective loss of weight, the satiety of Metrecal which satisfied hunger in a majority of patients, its simplicity of use—no calorie counting or menu planning required—and its palatability. No appetite depressants were required.

flexibility in use

When more than 900 calories are permitted, either the daily allotment of ½ pound of Metrecal may be increased or it may be used in conjunction with low-calorie foods. Metrecal may also be used for one or two meals a day or as the total diet two or three days a week.

easy to use

All patients do to make a full day's supply of Metrecal beverage is mix ½ pound of Metrecal powder with a quart of water. Or they may make a glassful at a time. For variety, Metrecal is available in Plain, Chocolate or Butterscotch flavors.

Weight-Control Guide for patient instruction is available from your Mead Johnson representative, or by writing to us, Evansville 21, Indiana.

references. (1) Antos, R. J.: Southwestern Med. *40*:695-697 (Nov.) 1959. (2) Tullis, I. F.: Initial Experience with a Simple Weight Control Formula, to be published. (3) Roberts, H. J.: Effective Long-Term Weight-Reduction—A Therapeutic Breakthrough, to be published.

Mead Johnson

Symbol of service in medicine

for morning sickness

brand of meclizine hydrochloride *

Bonine

DOSAGE:
One or two tablets give 24 hour protection. Administer at bedtime to prevent "next morning" sickness.

*Trademark

basic by every standard

effectiveness	✓	"Also, there are fewer therapeutic failures when patients are treated with . . . [BONINE] than with the other drugs."[11] ". . . [BONINE] is highly effective in relieving the nausea and vomiting of pregnancy, providing also the advantage of prolonged action. Thus, patients need not anticipate medication in the early morning hours when nausea is at its worst."[12]
safety	✓	". . . [BONINE] is a drug which is safe and nontoxic to both the mother and fetus and which is attended by a minimum of undesirable side effects."[13]
toleration	✓	"The incidence of side effects is very low and this agent exhibits less sedation and somnolence within therapeutic ranges than any of the effective antiemetic agents."[11] "Side effects were conspicuous by their rarity."[12]
convenience	✓	"The most striking advantage of . . . [BONINE] was that the administration of an effective dose only once daily, at bedtime, gave 24 hour protection, thus obviating the inconvenience and distress of repeating doses during the day."[14] ". . . [BONINE] is especially effective and has the advantages of a long duration of action (up to 24 hours) and a minimum of untoward side reactions."[11]
economy	✓	A single low-dosage drug providing therapeutic benefit at reasonable cost . . . contains no unnecessary added ingredients that increase cost . . . requires no extended-action tablet structure for prolonged effect.
experience	✓	The value of BONINE as an antinauseant has been well documented and is supported by six years of successful clinical use.[1-16]

only rarely does one drug meet so well the needs of one condition

REFERENCES: 1. Moyer, J. H.: M. Clin. North America, Mar., 1957, p. 405. 2. Lebherz, T. B., and Harris, J. H.: Obst. & Gynec. 6:606, 1955. 3. Mulherin, C. McL., and Bryans, C. I., Jr.: J. M. A. Georgia 45:46, 1956. 4. Bass, R. F.: Mississippi Doctor 32:176, 1954. 5. Seidner, H. M.: Illinois M. J. 109:20, 1956. 6. Charles, C. M.: Geriatrics 11:110, 1956. 7. Weil, L. L.: J. Florida Acad. Gen. Practice 4:9, No. 3, 1954. 8. Kinney, J. J.: J. M. Soc. New Jersey 53:128, 1956. 9. Semmens, J. P.: Obst. & Gynec. 9:586, 1957. 10. Conner, P. K., Jr., and Moyer, J. H.: GP 14:124, No. 5, 1956. 11. Daeschner, C. W., et al.: South. M. J. 49:1465, 1956. 12. Report of study by Army, Navy, Air Force Motion Sickness Team: J.A.M.A. 160:755, 1956. 13. Kean, B. H.: GP 20:112, No. 6, 1959. 14. Seppänen, A.: Geriatrics 14:457, 1959. 15. Hardman, E. F.: North Carolina M. J. 20:298, 1959. 16. Master, A. M.: New York J. Med. 58:2712, 1958.

SUPPLIED
BONINE Tablets, scored, 25 mg.
BONINE Chewing Tablets, mint-flavored, 25 mg.
BONINE Elixir, cherry-flavored, equiv. 12.5 mg./5 cc.

Professional Information Available on Request

PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. Brooklyn 6, N. Y. Pfizer *Science for the world's well-being™*

Proven

in over five years of clinical use

Effective

FOR RELIEF OF ANXIETY
AND MUSCLE TENSION

Outstandingly Safe

Simple dosage schedule produces rapid, predictable tranquilization without unexpected excitation. No cumulative effects, thus no need for difficult dosage readjustments. Does not produce ataxia, change in appetite or libido. No danger of hypotension, depression, Parkinson-like reactions, jaundice or agranulocytosis. Does not impair mental efficiency or normal behavior.

Miltown®

meprobamate (Wallace)

Usual dosage: One or two 400 mg. tablets t.i.d.
Supplied: 400 mg. scored tablets, 200 mg. sugar-coated tablets; or as MEPROTABS*—400 mg. *unmarked*, coated tablets.

CM-1903

WALLACE LABORATORIES/*New Brunswick, N. J.*

*TRADE-MARK

Whatever the indication,*
whatever degree of sedation desired,
a form of Nembutal will meet the need

ABBOTT

*OBSTETRICS—Eclampsia, Nausea and Vomiting, Amnesia.

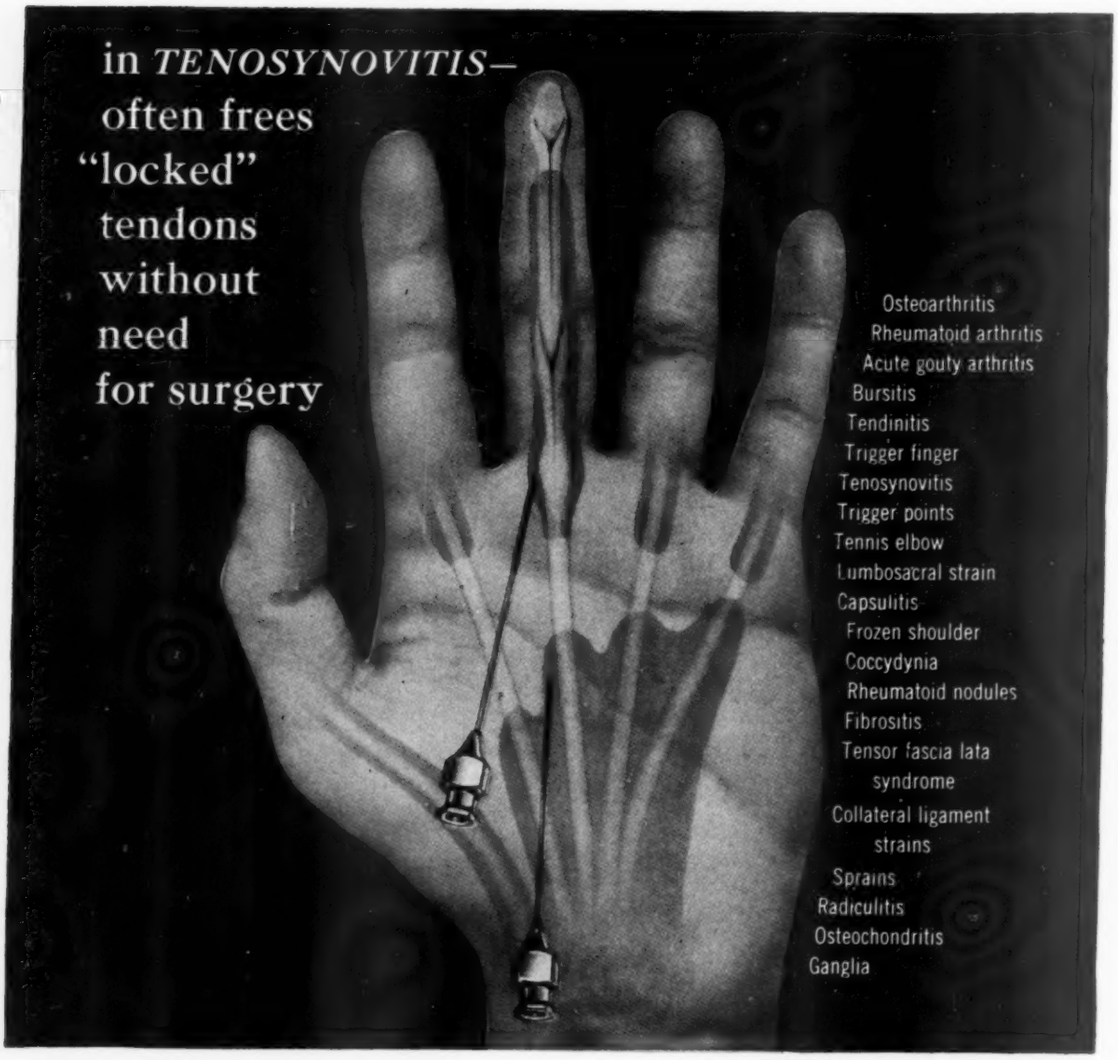
©ABBOTT LABORATORIES 004228

NEMBUTAL® (PENTOBARBITAL, ABBOTT)
(Nothing Faster, Shorter-Acting, Safer in Barbiturate Therapy)

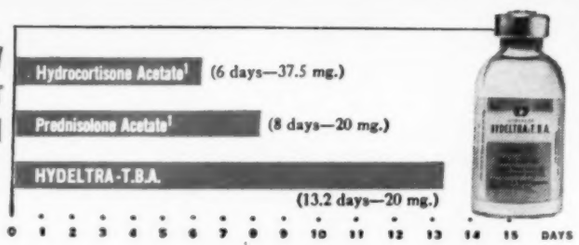
HYDELTRA®-T.B.A.

(Prednisolone *tertiary*-butylacetate, Merck)

for relief that lasts—longer



Anti-inflammatory effect lasts longer than that provided by any other steroid ester

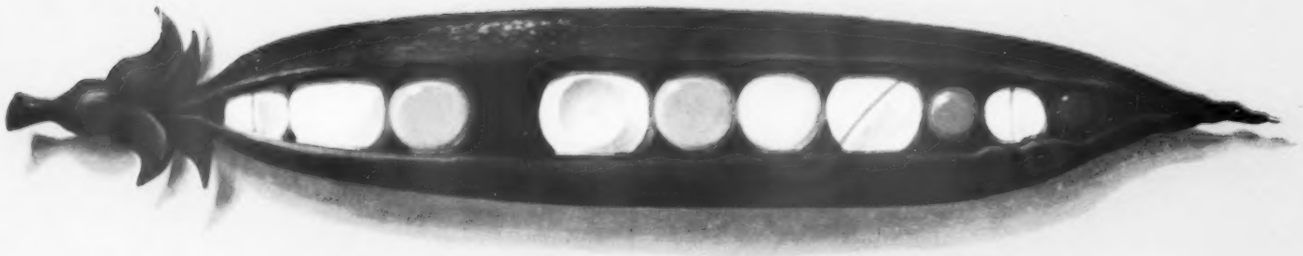

Hydrocortisone Acetate[1] (6 days—37.5 mg.)
Prednisolone Acetate[1] (8 days—20 mg.)
HYDELTRA-T.B.A.
(13.2 days—20 mg.)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 DAYS

Dosage: the usual intra-articular, intra-bursal or soft tissue dose ranges from 20 to 30 mg. depending on location and extent of pathology.

Supplied: Suspension 'HYDELTRA'-T.B.A.—20 mg./cc. of prednisolone *tertiary*-butylacetate, in 5-cc. vials.

MSD

MERCK SHARP & DOHME
DIVISION OF MERCK & CO., INC.
PHILADELPHIA 1, PA.



how does Mellaril *differ from other potent tranquilizers?*

Mellaril®

THIORIDAZINE HCl

provides highly effective tranquilization, relieves anxiety, tension, nervousness,

but is virtually free of such toxic effects as

jaundice
Parkinsonism
blood dyscrasia
dermatitis

SANDOZ

greater specificity of tranquilizing action results in fewer side effects

Virtual freedom of Mellaril from major toxic effects is due to greater specificity of tranquilizing action—divorced from such "diffuse" effects as anti-emetic action.

Mellaril

THIORIDAZINE HCl

specific, effective tranquilizer

"Thioridazine [Mellaril] is as effective as the best available phenothiazine, but with appreciably less toxic effects than those demonstrated with other phenothiazines....This drug appears to represent a major addition to the safe and effective treatment of a wide range of psychological disturbances seen daily in the clinics or by the general practitioner."*

Supply: MELLARIL Tablets, 10 mg., 25 mg., 100 mg.

SANDOZ

*Ostfeld, A. M.: Scientific Exhibit, American Academy of General Practice, San Francisco, April 6-9, 1959.

new nitrate offers new benefits for anginal patients

Fremont[1] notes that ISORDIL ". . . has been very effective in increasing the exercise tolerance of patients and also reducing the number of attacks of angina pectoris of the decubital type." Prophylactically and therapeutically, ISORDIL provides four distinctive advantages.

rapid onset—ready solubility of ISORDIL produces benefits within 15 to 30 minutes (not intended to replace emergency use of nitroglycerin).

prolonged action—benefits of ISORDIL persist for at least 4 hours per oral dose of 10 mg.

consistent effect—85 per cent of patients[2] treated have responded favorably to ISORDIL. In comparative studies, Sherber[3] found ISORDIL better than other therapy in 17 of 18 patients.

unusual safety—only reported side reaction: transitory, easily-controlled headache.

Electrocardiographic studies by Russek[4] clearly show that ISORDIL produces a more favorable balance between oxygen supply and demand following the Master two-step test. He concludes that "ISORDIL is a new and effective agent for therapy of angina pectoris."

Literature and professional samples available on request.

1. Fremont, R.E.: Personal Communication (Dec., 1959).
2. Summary of Case Reports on File, Ives-Cameron Company.
3. Sherber, D.A.: Personal Communication (Oct., 1959).
4. Russek, H.I.: Personal Communication (Oct., 1959).

TABLETS

ISORDIL*

Isosorbide Dinitrate, Ives-Cameron

IVES-CAMERON COMPANY • New York 16, N.Y.

*Trademark

LIVING...

. . . goes on much the same for the new diabetic on ORINASE*

There was a time when, for many patients, a diagnosis of diabetes mellitus meant abrupt and radical departure from a normal way of living. The constraint of rigid management often forced painful adjustments at an age when changes are not easily effected. With Orinase, however, three out of four diabetics can continue in their normal occupations, and family, social, and community activities are rarely impeded.

The majority of new diabetics past the age of 30 deserve a trial on Orinase . . . *first.*

*TRADEMARK, REG. U. S. PAT. OFF.—TOLBUTAMIDE, UPJOHN

Upjohn THE UPJOHN COMPANY KALAMAZOO, MICHIGAN

ORINASE

An exclusive methyl "governor" prevents hypoglycemia

90% of anxious, agitated and apathetic office patients calmed without drowsiness and with normal drive restored...

on one or two 0.25 mg. tablets b.i.d.:

This is the pattern of performance for

PERMITIL®

Fluphenazine dihydrochloride

■ In 608 patients with anxiety and anxiety-induced fatigue or depression, PERMITIL, administered in small daily doses of 0.5 mg. to 1 mg., produced significant improvement in 90%.*

■ PERMITIL is virtually free from side effects at recommended dosage levels.

■ Patients become calm without being drowsy and normal drive is restored.

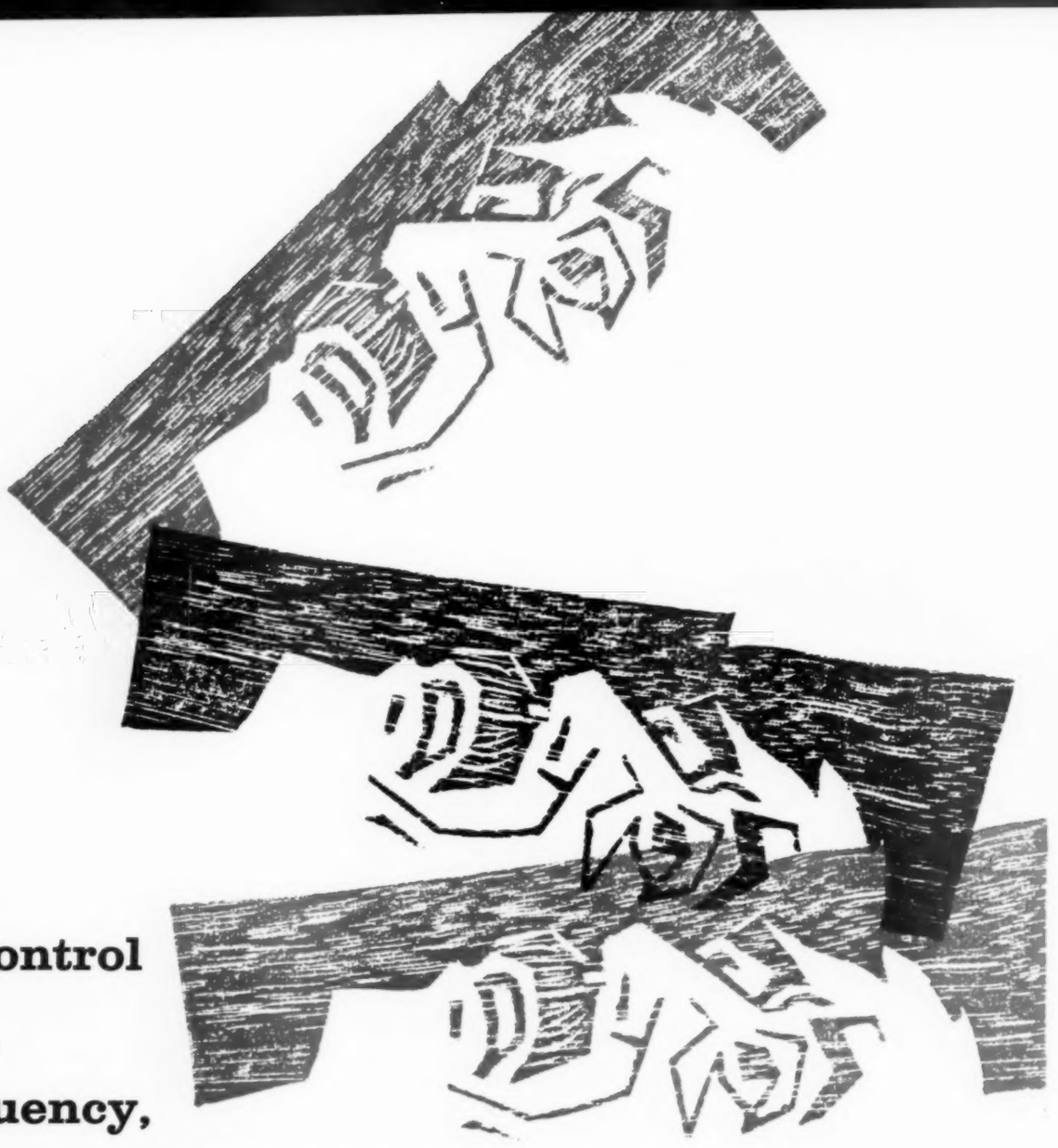
■ Onset of action is rapid; effect is prolonged. ■ PERMITIL does not potentiate barbiturates or non-barbiturate sedatives and can be used with impunity with such agents.

How to Prescribe PERMITIL: The lowest dose of PERMITIL that will produce the desired clinical effect should be used. The recommended dose for most adults is one 0.25 mg. tablet twice a day (taken morning and afternoon). Increase to two 0.25 mg. tablets twice a day if required. Total daily dosage in excess of 1 mg. should be employed only in patients with relatively severe symptoms which are uncontrolled at lower dosage. In such patients, the total daily dose may be increased to a maximum of 2 mg., given in divided amounts. Complete information concerning the use of PERMITIL is available on request.

Supplied: Tablets, 0.25 mg., bottles of 50 and 500.

*Recent compilation of case reports received by the Medical Department, White Laboratories, Inc.

White WHITE LABORATORIES, INC., *Kenilworth, New Jersey*



To control fear, frequency, and severity of anginal attacks

Equanitrate*

Meprobamate and Pentaerythritol Tetranitrate, Wyeth

EQUANITRATE helps control pain and accompanying anxiety in angina pectoris. It reduces the number and severity of attacks, increases exercise tolerance, and lessens nitroglycerin dependence.

A recent double-blind study† comparing meprobamate, a placebo, PETN, and EQUANITRATE states: "The best results . . . in both clinical and electrocardiographic response, were observed with a combination of meprobamate and pentaerythritol tetranitrate [EQUANITRATE]. . . ."

For further information on prescribing and administering EQUANITRATE see descriptive literature, available on request.

Wyeth Laboratories Philadelphia 1, Pa.

100 ANNIVERSARY Wyeth®

A Century of Service to Medicine

†Russek, H.I.: Am. J. Cardiol. *3*:547 (April) 1959.

*Trademark

Supplied: **EQUANITRATE 10** (200 mg. meprobamate, 10 mg. pentaerythritol tetranitrate), white oval tablets, vials of 50. **EQUANITRATE 20** (200 mg. meprobamate, 20 mg. pentaerythritol tetranitrate), yellow oval tablets, vials of 50.

COUMADIN BIBLIOGRAPHY

Published studies on anticoagulant therapy with COUMADIN

1. Friedman, B.: The use of anticoagulants in the treatment of coronary and cerebral vascular disease, J. Tennessee M. A. 52:171, May, 1959. 2. Shapiro, S.: Long term therapy of coronary arteriosclerosis, Angiology 10:126, Apr., 1959. 3. Vastola, E. F., and Frugh, A.: Anticoagulants for occlusive cerebrovascular lesions, Neurology 9:143, Mar., 1959. 4. Toohey, M.: Clinical experience with warfarin sodium, Brit. M. J. 2:892, Oct. 11, 1958. 5. Porter, R. R.; Richardson, D., and Mauck, H. P., Jr.: Clinical experiences with the anticoagulant warfarin sodium ("Coumadin Sodium"), Virginia M. Month. 85:465, Sept., 1958. 6. Trumble, E. A.: Clinical experience with intramuscular warfarin sodium (Coumadin Sodium), Surg. Gynec. & Obst. 107:303, Sept., 1958. 7. Goodman, D. H.: Early clue to visceral carcinoma—hemorrhage after intravenously given warfarin, J.A.M.A. 166:1037, Mar. 1, 1958. 8. Shapiro, C. M.; Lisker, R.; Lichtman, A. M., and Josephson, A. M.: Comparative clinical study of Coumadin Sodium and Dicumarol in patients with thromboembolic diseases, Am. Heart J. 55:66, Jan., 1958. 9. Yarrow, M. W.; Baer, S.; Kravitz, C., and Markson, V.: A preliminary report on sodium warfarin (Coumadin), J. Albert Einstein M. Center 5:205, June, 1957. 10. Baer, S.; Yarrow, M. W.; Kravitz, C., and Markson, V.: Clinical experiences with warfarin (Coumadin) sodium as an anticoagulant, J.A.M.A. 167:704, June 7, 1958. 11. Fremont, R. E., and Jagendorf, B.: Clinical observations on use of warfarin (Coumadin) sodium, a new anticoagulant, J.A.M.A. 165:1381, Nov. 16, 1957. 12. Shapiro, S., and Ciferri, F. E.: Intramuscular administration of the anticoagulant warfarin (Coumadin) sodium, J.A.M.A. 165:1377, Nov. 16, 1957. 13. Nicholson, J. H.: Clinical experiences with anticoagulants: A comparison of Coumadin (warfarin) Sodium and Dicumarol (bishydroxycoumarin), Angiology 8:456, Oct., 1957. 14. Kerrin, H. F.; Guidot, J., and Wilhelm, S. K.: Clinical experiences with the anticoagulant Coumadin (warfarin) Sodium, Angiology 8:302, June, 1957. 15. Goodman, D. H.: Experience with a new anticoagulant Coumadin® (warfarin) Sodium, Arizona Med. 13:389, Oct., 1956. 16. Nicholson, J. H., and Leavitt, T., Jr.: Coumadin (warfarin) Sodium: A new anticoagulant, New England J. Med. 255:491, Sept. 13, 1956. 17. Clatanoff, D. V., and Meyer, O. O.: Further observations on use of warfarin sodium in anticoagulant therapy, A.M.A. Arch. Int. Med. 97:753, June, 1956. 18. Freeman, D. J., and Meyer, O. O.: Rectal administration of warfarin (Coumadin) sodium. Sodium [3 (2-acetonyl-benzyl)-4 hydroxycoumarin], Proc. Soc. Exper. Biol. & Med. 92:52, May, 1956. 19. Pollock, B. E.: Clinical experience with Coumadin Sodium, a new anticoagulant drug, Angiology 6:506, Dec., 1955. 20. Pollock, B. E.: Clinical experience with warfarin (Coumadin) sodium, a new anticoagulant, J.A.M.A. 159:1094, Nov. 12, 1955. 21. Shapiro, S.: The hypoprothrombinemia-inducing activity of warfarin sodium (Coumadin® Sodium), J. Kansas M. Soc. 55:687, Dec. 1954. 22. Clatanoff, D. V.; Triggs, P. O., and Meyer, O. O.: Clinical experience with coumarin anticoagulants warfarin and warfarin sodium, A.M.A. Arch. Int. Med. 94:213, Aug. 1954. 23. Shapiro, S.: Warfarin sodium derivative: (Coumadin® Sodium): An intravenous hypoprothrombinemia-inducing agent, Angiology 4:380, Aug., 1953.

Other published literature referring to Endo's COUMADIN

24. Escudero, J.; McDevitt, E., and Wright, I. S.: Dicumarol, Coumadin, Marcumar and Tromexan: Comparative study of their action on the clot as registered by the thrombelastogram, Circulation 20:405, Sept., 1959. 25. Hillier, W. F., Jr.: Little strokes, West Virginia M. J. 55:259, Aug., 1959. 26. Wright, I. S.: Strokes; the present status of diagnosis and treatment, Postgrad. Med. 25:549, May, 1959. 27. Little, J. R.: Purpura fulminans treated successfully with anticoagulation, J.A.M.A. 169:104, Jan. 3, 1959. 28. Link, K. P.: The discovery of Dicumarol and its sequels, Circulation 19:97, Jan., 1959. 29. Kleiner, I. S., and Orten, J. M.: Human Biochemistry, ed. 5, Mosby, St. Louis, 1958, pp. 211-212. 30. Meyer, O. O.: Use of anticoagulants in the treatment of coronary artery disease, Postgrad. Med. 24:110, Aug., 1958. 31. Toohey, M.: Long-term anticoagulant therapy for coronary thrombosis, Brit. M. J. 2:473, Aug. 23, 1958. 32. McDevitt, E.; Wright, I. S., and Foley, W. T.: Present status of anticoagulant treatment of cerebral vascular lesions, M. Clin. North America, May, 1958, p. 587. 33. Barker, N. W.: Fundamentals of anticoagulant therapy, Minnesota Med. 41:252, Apr., 1958. 34. Grant, W. G., and MacMillan, R. L.: Experience [illegible] coagulant therapy, Canad. M. A. J. 78:317, Mar., 1958. [illegible] Treatment of peripheral arterial disease, Dis. Chest 33: [illegible] Brotman, I.: Anticoagulants in myocardial infarction, Am. [illegible] 1958. 37. Toohey, M.: Anticoagulants in myocardial infar[illegible] Feb. 1, 1958. 38. Editorial: Warfarin, Virginia M. Mont[illegible] 39. Friedberg, C. K.: Recent advances in coronary heart[illegible] agement, New York J. Med. 57:3643, Nov. 15, 1957. [illegible] sodium as a long-term anticoagulant, M. Science 2[illegible] J. H., and Oglesby, P.: Long-term anticoagulant thera[illegible] 105:61, July, 1957. 42, Ware, A. G., and Stragnell, [illegible] Elimination of some commonly occurring pitfalls, Ann[illegible] 1957. 43. Briner, D. H.: A study of 235 cases of myo[illegible] Osteopath. A. 56:349, Feb., 1957. 44. Cosgriff, S. W.: [illegible] coronary artery disease, Am. J. Chron. Dis. 4:402, C[illegible] S. A., and Aird, R. B.: M. Clin. North America, Sept., 1[illegible] B. E.: The early management of myocardial infarction, [illegible] 1956. 47. Shapiro, S.: Anticoagulant therapy, Surg. G[illegible] 1956, p. 469. 48. Wright, I. S.: Present status of ant[illegible] treatment of myocardial infarction; the use and miss[illegible] evaluation of new anticoagulants, their indications ar[illegible] 43:942, Nov., 1955. 49. Shapiro, S. (editorial): The a[illegible] prothrombinemia-inducing drugs, Angiology 6:498, Oc[illegible] Principles of anticoagulant therapy and their applicat[illegible] ica, Jan., 1955, p. 227. 51. Shapiro, S., and Spi[illegible] (ascorbic acid plus "P" factors) in drug-induce[illegible] hypop[illegible] ology 5:64, Apr. 1954.

Coumadin is the original and only warfarin sodium responsible for establishing this drug as "the best anticoagulant available today"[30]

(over 50 published papers since 1953)

COUMADIN®

FOR ORAL, INTRAVENOUS OR INTRAMUSCULAR USE

SODIUM

IN MYOCARDIAL INFARCTION AND OTHER THROMBOEMBOLIC DISORDERS

SUPPLIED: ***Oral***—scored tablets, 2 mg., 5 mg., 7½ mg., 10 mg., 25 mg. ***Parenteral***—single injection units, consisting of one vial, 75 mg., and one 3-cc. ampul Water for Injection.

COUMADIN (warfarin) Sodium is manufactured under license from the Wisconsin Alumni Research Foundation.. *clinically established by Endo.*

Complete Information and Reprints on Request

ENDO LABORATORIES
Richmond Hill 18, New York

Over 60,000,000 doses of Coumadin administered to date

in cardiac edema of varying severity

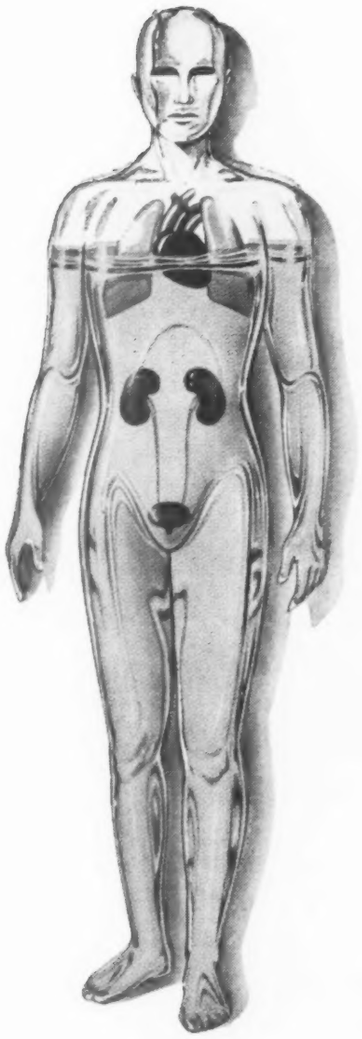
weight loss ranged from 4 to 45 lbs. on

HYDRODIURIL®

HYDROCHLOROTHIAZIDE

increased potency—without corresponding increase in side effects

Sackner, M. A., Wallack, A. A. and Bellet, S.: Am. J. M. Sc. 237:575, (May) 1959.



"The severity of the congestive heart failure . . . was as follows: Class IV (9 patients), Class III (5 patients), and Class II (1 patient).". . ."Weight loss ranged from 4 to 45 pounds over a period of 3 to 17 days with an average of 2.4 pounds a day."

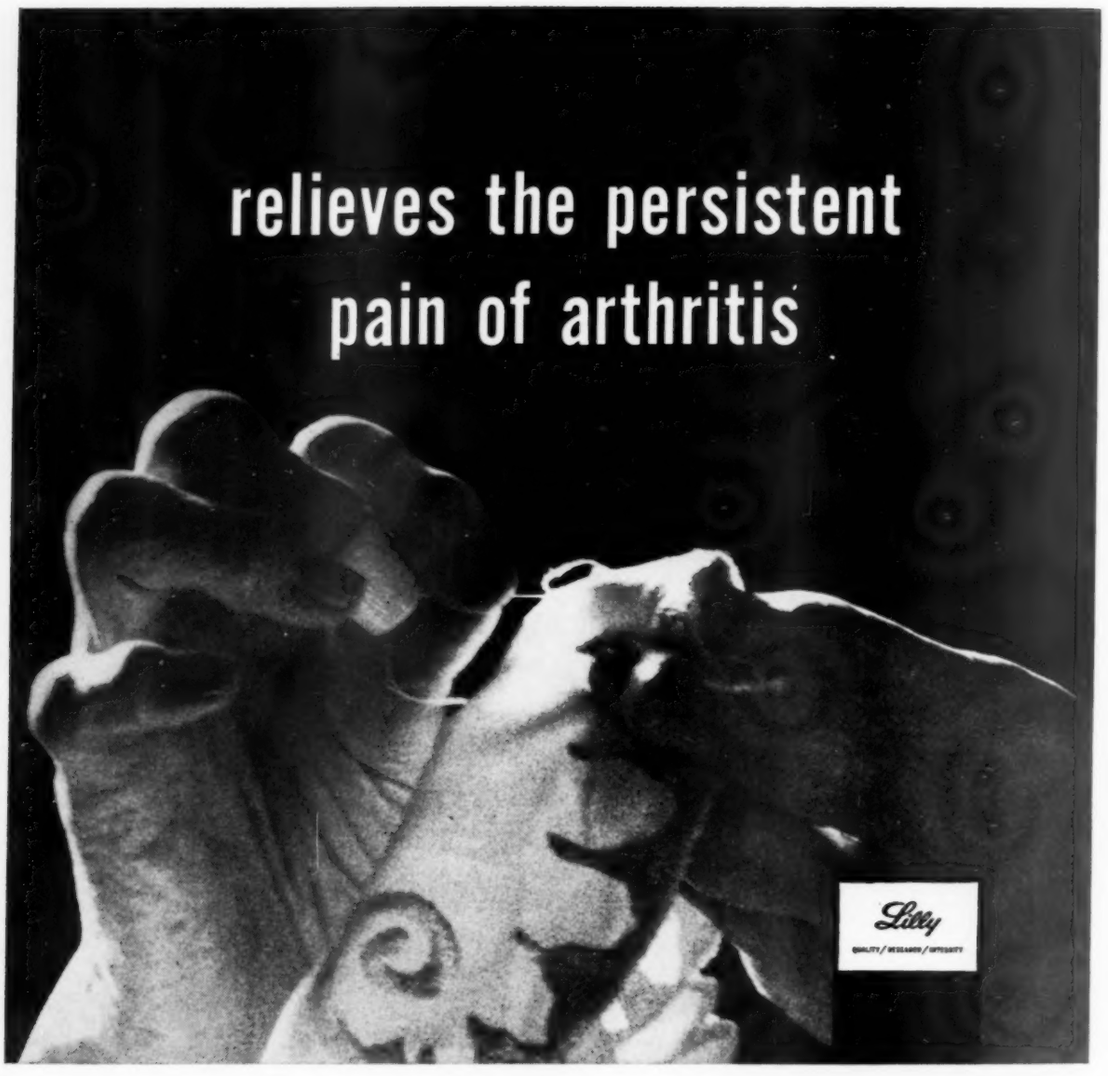
DOSAGE: One or two 50 mg tablets of HYDRODIURIL once or twice a day.

SUPPLIED: 25 mg. and 50 mg. scored tablets HYDRODIURIL (Hydrochlorothiazide) in bottles of 100 and 1,000.

HYDRODIURIL is a trademark of Merck & Co., INC.

Additional information on HYDRODIURIL is available to the physician on request.

MSD MERCK SHARP & DOHME
Division of Merck & Co., INC. Philadelphia 1, Pa.



DARVON® COMPOUND

(dextro propoxyphene and acetylsalicylic acid compound, Lilly)

Darvon Compound combines the analgesic action of Darvon® with the anti-inflammatory and antipyretic benefits of A.S.A.® Compound. When inflammation is present, Darvon Compound reduces discomfort to a greater extent than does either analgesic given alone.

Usual dosage: 1 or 2 Pulvules® three or four times daily.
Also available: Darvon, in Pulvules of 32 and 65 mg.

Darvon® (dextro propoxyphene hydrochloride, Lilly)
A.S.A.® Compound (acetylsalicylic acid and acetophenetidin compound, Lilly)

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U. S. A.

020220

The American Journal of Medicine

VOL. XXVIII MAY, 1960 No. 5

Symposium on Leukemia

Introduction

LEUKEMIA continues to be a baffling disease. While it is spoken of as a single disease, it may in reality be several different disorders; and there is no proof that it represents more than a response on the part of one of the blood forming organs to some stimulating or inciting agent or agents. Definite advances have been made in our attack on it during the past quarter of a century but we are still far from any definitive answer to the problems presented by it.

The etiology remains obscure but some progress in elucidation has been made. There can no longer be any doubt about the leukemogenic effect of radiation in both man and animals. Serious consideration has been given to virus or viruses as possible factor(s) and this may be the inciting cause of the disorder in certain instances. Metabolic or deficiency states still have their supporters. The most generally accepted theory is that leukemia is neoplastic in the sense that the proliferation of the particular leukocytic tissue is progressive, uncontrolled and purposeless. The great importance of heredity must not be overlooked. In the long run it is the balance between hereditary factors and environment that keeps us on the normal path.

Great strides have been made in the treatment of leukemia during this interval. While it is true that no cure has been found for leukemia, the course of the disease can be greatly modified by chemotherapy. The most striking and spectacular effects have appeared in acute leukemia but chronic leukemia also may be affected markedly. The development of drugs for the treatment of leukemia is based primarily on knowledge of the biochemistry of cell growth but the trial of various drugs in leukemia is largely empirical. Nevertheless, it should be recognized that many of the most important discoveries in medicine have been of this nature and not the result of a planned scientific program. The marked changes in the symptomatology, metabolism, peripheral blood and hematopoietic tissues caused by drugs lead us to hope and expect that we are close to uncovering something that will cure the disorder. Certainly, the effects produced by these agents have given a tremendous impetus to the study of basic abnormalities in leukemia.

The introduction of the electron microscope into clinical medicine has opened up large and new fields in morphology, as has been so well shown by Bessis [1]. A whole new field awaits exploration but whether this will result in the discovery of a means for eradicating the disease can well be doubted However, it should improve our ability to differentiate acute and subacute leukemias.

Biochemical studies of the leukocytes have resulted in many new and worth while discoveries. A new set of criteria is developing for delineation of the leukemias. The best known and most reliable enzyme change relates to alkaline phosphatase. The marked reduction of this enzyme in chronic myeloid leukemia has been well documented, so much so that one thinks twice before making this diagnosis in the presence of normal or elevated alkaline phosphatase levels of the white blood cells. The

[1] BESSIS, M. Phase contrast microscopy and electron microscopy applied to the blood cells. *Blood*, 10: 272, 1955.

internal composition of the white blood cell is still incompletely understood and demands much further study. More complete delineation of the substances within the normal and abnormal white blood cells could give the clue to the proper drug or drugs for the eradication of leukemia.

Finally, investigation of the dynamics and physiology of the white blood cells has gained considerable momentum within the past several years. The rate of utilization and the reserve of the white blood cells are receiving intensive study. Some of the more recent observations indicate that we may have to change our previous concepts. For example, the study of DNA synthesis in a population composed largely of blasts shows that the turnover is very slow. One conclusion from this is that these cells are *not* dividing at a rapid rate but at a slow rate. If this is so, the great increase in cells in such conditions is due to long life rather than rapid production. This, of course, does not explain the apparent failure of maturation in acute leukemia. Much imagination and ingenuity of investigators is demanded by experimentation in this field. It would seem that continued study in this area would lead to exciting discoveries, some one of which may give us the key to leukemia.

JOHN S. LAWRENCE, M.D.
Department of Medicine,
University of California
Medical Center,
Los Angeles, California

Radiation Leukemogenesis*

An Analysis of the Problem

E. P. CRONKITE, M.D., WILLIAM MOLONEY, M.D. *and* V. P. BOND, M.D., PH.D.
Boston, Massachusetts

THE carcinogenic effect of radiation was recognized shortly after Konrad Roentgen's discovery of x-rays. In 1911 the first cases of leukemia attributed to x-ray and radium exposure were reported by Von Jagie et al. [*1*]. However, leukemogenesis constitutes only a small fraction of the total problem of radiation hazard, which concerns not only carcinogenesis but also such effects as life shortening, decreased fertility, cataracts and genetic damage.

On March 1, 1954, following the detonation of a nuclear bomb, a large scale fallout occurred [*2*] and several hundred persons were accidentally exposed to considerable amounts of radioactivity. This event forcibly drew attention to the fact that testing of nuclear devices contaminated the atmosphere and resulted in prompt and prolonged deposition of fallout products over the entire surface of the earth. The problem has been scrutinized carefully by national and international groups [*3–8*]. Whereas the primary purpose of these investigations was to determine the hazard from fallout and nuclear radiations, all these reports serve to emphasize that in the more highly educated and technologically advanced parts of the world, the major source of radiation exposure is derived from the medical use of x-rays.

Lewis [*8*] analyzed several sets of human data, in which exposure to radiation apparently was associated with leukemogenesis in man. From his analysis, and using several assumptions which he considered reasonable and consistent with the data, he concluded that exposure to radiation even in small doses (fraction of a rad) may produce leukemia in some individuals so exposed (approximately two cases per year per 10^6 exposed per rad).

There is no question that exposure of the population to ionizing radiation from medical and industrial uses of a variety of radiation sources will continue to increase [*8*]. If the anticipated exposure to radiation in the present and future is truly hazardous then it is mandatory that all members of the health professions and all users of radiation be cognizant of the potential hazards involved. The primary purpose of this paper is to re-examine, in the light of present knowledge, the several sources of human data bearing on the problem in an effort to determine to what degree predictions of the incidence of leukemia following exposure of human populations to ionizing radiation are possible.

LEUKEMIA IN SURVIVORS OF THE JAPANESE ATOMIC BOMB EXPLOSION

Annual Incidence and Age Susceptibility. The data which are presented and analyzed have been abstracted from the excellent reports of the Atomic Bomb Casualty Commission (ABCC) [*9–14*]. The first case of leukemia was noted in October 1945 but in all probability occurred too soon after exposure to have been induced by radiation. In May and July 1947 in Hiroshima and in January and November 1947 in Nagasaki, cases of leukemia were encountered among survivors of the atomic bomb explosions. A definite increase in leukemia thus seemed to commence about one and a half years after the bombings. However, the minimum latent period may actually be shorter since adequate studies were not feasible due to the chaotic conditions in the bombed cities prior to this time. The greatest number of leukemia cases, according to year of onset, was reported in 1951 and 1952; it is evident that the appearance of leukemia cases reached a maximum in this period. However, the number in 1957 apparently increased over

* From the Medical Research Center, Brookhaven National Laboratory, Upton, New York, and the Clinical Laboratories, Boston City Hospital, Boston, Massachusetts. This research study was supported by the U. S. Atomic Energy Commission.

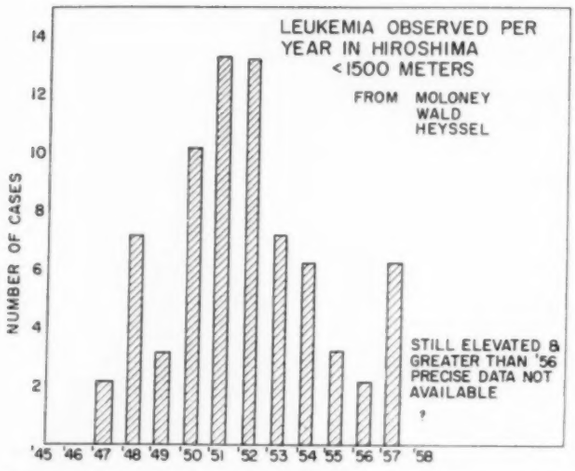

LEUKEMIA OBSERVED PER YEAR IN HIROSHIMA
<1500 METERS
FROM MOLONEY WALD HEYSSEL
NUMBER OF CASES
STILL ELEVATED & GREATER THAN '56 PRECISE DATA NOT AVAILABLE
'45 '46 '47 '48 '49 '50 '51 '52 '53 '54 '55 '56 '57 '58

FIG. 1. Histogram of cases of leukemia found per year at Hiroshima between 1945 and 1958.

that seen in 1956. The high incidence in 1951 and 1952 may be bona fide or it may be the result of the effectiveness and enthusiasm of the investigators at finding leukemia cases during this interval. The annual incidence of cases from Heyssel et al. [*14*] is shown in Figure 1. For similar reasons the incidence prior to 1951 may well be underestimated. The modal incubation time is approximately five years. It has been deduced by Cobb et al. [*15*], on the basis of the Japanese and the spondylitic data, that probably 90 per cent of the cases will occur within approximately ten years after exposure. On the basis of the Japanese data, the Cobb analysis [*15*] and animal data to be discussed later, it appears that an assumption that the incidence of leukemia will continue at a constant rate throughout duration of life cannot be justified.

The Determination of Effective Dose at Hiroshima and Nagasaki. The estimation of the effective dose at Hiroshima and Nagasaki is complicated. The nuclear devices used in the two cities were significantly different in respect to the proportion of gamma radiation to fast neutrons. Since it has been commonly assumed by many physicians and biologists that the dose of radiation from a nuclear device can be easily computed, the subject warrants more than a passing comment. The dose of radiation, of course, was not measured at the time of detonation of the bombs, and certain data are essential to compute accurately the dose distance curve. For example, information as to the precise yield of the device, its precise location, the gamma neutron ratio and air density at the time of detonation are minimum essentials to estimate the air dose at any point. The dose delivered to people depends upon knowledge of their precise position and degree of attenuation by shielding material. In the mixed gamma neutron exposures such as occurred at Hiroshima, the relative biological effectiveness (RBE) factor for the neutron becomes an important item.

Since the RBE of neutrons on cells is equal to or greater than gamma rays for the same amount of energy deposited in the cell, the effect of this factor on the over-all response must be evaluated. In Figure 2 the influence of arbitrarily changing the RBE of neutrons from 0 to 2 on the dose effect relationship is shown. As the RBE is decreased the "rem" dose decreases for the same effect, since neutrons contribute less and less to the total dose. Thus the RBE used for neutrons significantly influences the slope of the leukemia incidence curve versus total dose. Experimentation, amply reviewed [*16*] shows that the RBE varies not only with LET but also with the biological effect observed. The RBE for leukemia in man, even when the neutron dose is equally deposited in all sensitive cells, is not known. Any number used is purely a guess. In addition, in tissue, with its high hydrogenous content, attentuation and thermalization is rapid so that there is a marked decrease in dose with depth in tissue. This is not compensated for appreciably by an isotropic beam. Thus on depth dose considerations alone, an RBE determined in the mouse should not be used for man. Simple addition of the gamma air rad to first collision neutron dose does not necessarily estimate the "effective dose." The RBE of neutrons for acute effects in man probably is less than 1 [*17*] and thus the RBE for leukemia induction in man may well be less than 1.

The extensive shielding study under way in Japan at the ABCC for both Hiroshima and Nagasaki* may aid in clarifying these problems.

The Dose Effect Relationship for Incidence of Leukemia in Hiroshima Through 1957. Heyssel and associates [*14*] have recently published extensive and valuable documentary data on the incidence of leukemia in Hiroshima. Their publication permits one to analyze the data as may be desired. Figure 3 is a replot of their

* This work is underway at the Hiroshima Atomic Bomb Casualty Commission in collaboration with Oak Ridge National Laboratory; it is sponsored by the National Academy of Sciences and the U. S. Atomic Energy Commission.

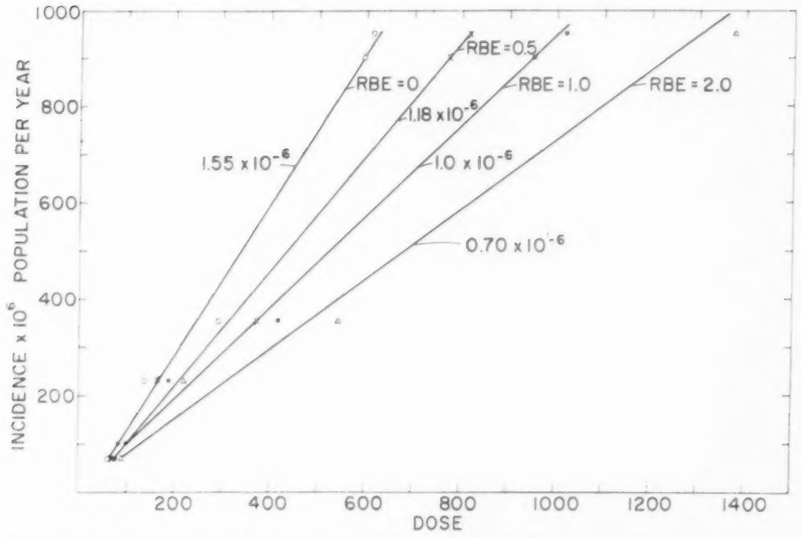


FIG. 2. Curves for incidence of leukemia in Hiroshimo versus dose of radiation as the neutron RBE is changed from 0-2 for cumulative cases through 1957.

data showing the cumulative cases through 1957 against the dose of radiation. The confidence limits of the incidence per million population is shown by the vertical lines at each point. In the Heyssel publication the dose was shown as a single point. The range in the systematic error of the dose was not shown. In Figure 3 the estimated range of the "systematic error" for the dose is extracted from the computations by York in the Heyssel paper and is plotted for two of the incidence points. This provides a rectangular area in which the real dose effect point may lie within these limits of error. Omission of the systematic error of the dose tends to give an apparent stability to the dose point. This wide error in the estimate of the dose is not a statistical estimate, but stems from the inevitable systematic errors that are inherent in computing dose. With the exception of the azimuth, a systematic error would cause all doses to be altered in the same direction. Hence the effect would primarily be one of changing the slope of whatever curve may be drawn between the points. It would be unlikely to change the shape unless the major error was one of position, primarily horizontal, of the bomb. If the numbers were large enough in a closed population, with adequate dosimetry, it would be of considerable interest to know the incidence in a given ring of acute symptoms, such as leukemia and a fall in leukocytes, as a function of the azimuth. This would throw additional light on the reliability of dose estimates. Despite the

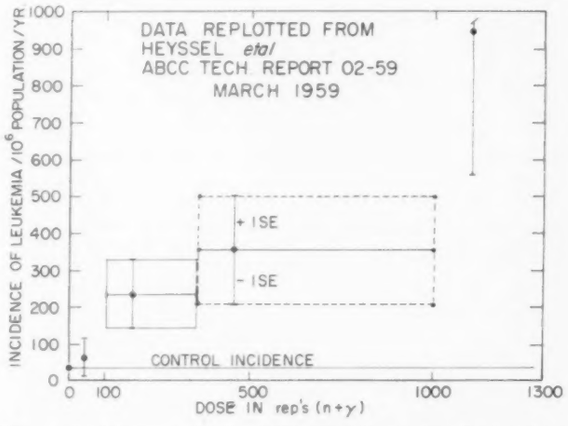


FIG. 3. Incidence of leukemia versus dose of radiation for period of 1945 through 1957 showing statistical error of the estimate and systematic error of the dose.

uncertainties inherent in the data, for purpose of predictions the points between the lowest and the highest doses can be treated as if they were linear. The slope is not known with precision, but for a general guide the incidence of leukemia per rad, measured outside Japanese homes, per million people at risk per year, following a single exposure of this type is about $1/10^6$ for at least the first ten to fifteen years after exposure. Since neither the dose within the homes nor the absorbed dose is known adequately, it is not possible to estimate the incidence per unit of absorbed dose; however the incidence would be greater than that recorded herein.

LEUKEMIA IN PERSONS WITH SPONDYLITIS EXPOSED TO RADIATION

The development of leukemia in persons with spondylitis exposed to radiation has been the subject of repeated analyses in the United Kingdom report [*4*] and in a series of articles by Court-Brown and Doll [*18,19*]. These investigators indicate that the relationship of dose to leukemia may be linear. They conclude in the analysis of the data that there may not be a threshold dose and that the dose which would double the incidence of leukemia lies between 30 and 50 r. They estimate as of the order of 1×10^{-6} and 2×10^{-6} the probability of developing leukemia per year per rad, averaged over the whole red bone marrow system. However, it is of interest that when they consider the spontaneous incidence and compute the confidence limits of the X intercept the range for a threshold lies between 0 and approximately 400 rad. Additional difficulties in evaluating these data involve certain peculiarities of the group: (1) all adult males, (2) inflammatory bone disease, (3) doses are large and delivered only to the spinal area.

THE LEUKEMOGENIC HAZARD OF PELVIMETRY

Stewart et al. [*20*] have reported an apparent increase in the incidence of leukemia in infancy due to diagnostic radiation in utero. Ford et al. [*21*] have published similar observations. However, the data of others [*22–24*] do not support necessarily the conclusions of Stewart and Ford et al. Hemplemann et al. [*25,26*] point out that the choice of controls, which differed in these retrospective surveys, may be a crucial factor. Obviously, further information is needed before the hypothesis that diagnostic x-ray produces an increased incidence of leukemia in infancy from exposure of the fetus can be accepted. Moreover, the situation with fetal exposure to x-ray differs considerably from diagnostic radiation in adults. The fetus receives whole body radiation, the x-ray dose from pelvimetry may be considerably higher than from the usual diagnostic x-rays, and the factor of biologic sensitivity of fetal tissues should be considered.

It is imperative to establish whether pelvimetry is leukemogenic or not. Even though the doses to the fetus are not known with any certainty, the dose obviously is small. If leukemia does result from pelvimetry, it may indicate that the fetus is extremely sensitive or that in reality there is no threshold. It is essential to carry out further studies along these lines. If pelvimetry induces leukemia, and there is a threshold, this threshold, if it exists, becomes vanishingly small.

THYMIC RADIATION AND LEUKEMIA

In 1955 Simpson et al. [*25,26*] pointed out that there was a significant increase in the incidence of leukemia among children who had received x-ray therapy to an enlarged thymus gland. Subsequently these studies were extended to include children treated with radiation for other conditions; it was reported that in children treated for pertussis and lymphoid hyperplasia there was a greater number of deaths from leukemia. No cases of leukemia were discovered among children treated superficially for benign lesions. In a smaller series of cases reported by Snegeriff [*27*] there were no cases of leukemia in children who received x-ray therapy for an enlarged thymus gland. While Manning et al. [*28*] found no differences in the per cent of leukemia occurring in children given radiation therapy over the thymus compared to a control group, Polhemus and Koch [*23*] in their series noted a history of radiation therapy to the thymus in 4.3 per cent in patients with leukemia compared to 0.8 per cent in control subjects. Conti and Patton [*29*] reported that in 1,564 children given x-ray therapy to a normal sized thymus no cases of leukemia were found in an eleven to eighteen year follow up. Since an entirely satisfactory control group of children with enlarged thymus glands who did not receive radiation therapy is unavailable, final decision on the role of radiation and enlarged thymus glands to the increased incidence of leukemia must await the outcome of further observations. Whereas there is some question about leukemia, the development of thyroid carcinoma in children receiving radiation therapy to the thymus appears to be on firmer ground. Because of uncertainties in dose, the port size, and the precise area radiated, it appears impossible to treat these data quantitatively and to deduce any realistic yield of leukemia per rad of exposure to the thymic area.

ADDITIONAL REPORTS OF LEUKEMIA FOLLOWING EXPOSURE TO RADIATION

A number of cases of leukemia following exposure to radiation have been reported, and the heterogeneity of the material has been pointed out [*30*]. These cases are discussed under the following headings:

X-ray Therapy. From the epidemiological point of view, sporadic case reports of radiation-induced leukemia have been of little statistical value. In reviewing reports of leukemia following x-ray therapy, only seventy-three patients to date were found to have a reasonable association to radiation exposure. Of this group, fifty-five occurred in patients receiving x-ray therapy for spondylitis (forty-nine of these have been discussed earlier). In eighteen other cases of leukemia following x-ray therapy for conditions other than spondylitis, the patients were treated initially for a variety of diseases, including malignant and benign tumors, skin disorders, asthma and arthritis. Although of little epidemiological value, these cases have been fairly well documented from the clinical, hematological and radiation-dose aspects. All such cases deserve to be reported in detail since they constitute one of the few sources of information on the latent period, type of leukemia and other features of radiation-induced leukemia in man.

Thorium Therapy. To date nine cases of leukemia following intravascular administration of thorium compounds for either diagnostic or therapeutic indications were found recorded in the literature. Estimation of the dose of radiation has been difficult. The number of cases is small, and the long latent period and other features complicate the analysis.

I^{131} Therapy. With the increasing use of this radioactive isotope in clinical medicine the reports of nine cases of leukemia following therapeutic and diagnostic use of I^{131} have been of special concern. All nine cases have been well documented from the clinical, hematological and the radiation standpoint. In five instances large therapeutic doses of I^{131} were given and the induction of leukemia by radiation appears a reasonable assumption. However, in four cases of hyperthyroidism relatively small amounts of I^{131} were employed. Before radiation-induced leukemia can be attributed to these low dose levels, additional evidence must be obtained. The possible role of metabolic factors and antithyroid drugs should be considered in these patients.

Diagnostic Radiations. A number of prospective surveys of large groups of subjects exposed to a variety of low level x-ray and other forms of ionizing radiation are in progress in the United States of America and in other parts of the world. Until such information, based on accurate radiobiological, hematological and epidemiological data is available, statements that diagnostic radiation cause leukemia are unwarranted.

Occupational Exposure. Leukemia has been reported in radiologists, nurses, technicians and physicists. To date there have been references to twenty-five cases in this category from all over the world. However, in a review of this material [*30*] only twelve case reports provided adequate data for analysis. It should be noted that these cases occurred at a time when there was little protection to individuals exposed for prolonged periods to x-rays, radium and radioactive substances. In addition to these twenty-five cases, an increased incidence of leukemia among radiologists has been established statistically by several investigators [*32,33*]. March [*34*] in 1950 presented an analysis of physicians' deaths from 1929 to 1948. He found ten cases of leukemia among 299 deaths of radiologists, a rate of 4.69 per cent. In non-radiologists this rate was 0.48 per cent. One of us (W. C. M.) in an attempt to carry out a similar analysis from 1949 to 1958, examined the obituaries of 28,496 physicians appearing in the *J. A. M. A.* There were 23,393 diagnoses available and among 296 deaths in radiologists eleven were attributed to leukemia. This would indicate a rate of 3.71 per cent. These data are not suitable for analysis since all deaths were not recorded and from 1949 through 1957 nearly 25 per cent of the diagnoses were not available. However, as noted by others, the indications are that the death rate from leukemia in all physicians rose slightly in the past nine years (but not as much as in the white male population of similar age groups). However, the rate among radiologists fell from 4.7 per cent to 3.71 per cent.

Obviously, such retrospective studies are open to serious criticisms and are of limited value. Until more data are available discussions on the leukemogenic effects of low level radiation are speculative.

RADIATION LEUKEMOGENESIS IN ANIMALS

Since the initial discovery by Krebs et al. [*35*] that radiation can induce leukemia in animals the problem has been considered in great detail by Furth et al. [*36,37*], Kaplan et al. [*38,39*] and Mole [*40*]. The extensive work of these and other investigators has shown that the induction of leukemia in animals is complex indeed. Shielding of hemopoietic tissues diminishes the yield of

TABLE 1
ADEQUATELY DOCUMENTED CASES OF LEUKEMIA ATTRIBUTED TO RADIATION EXPOSURE
Collected from the World Literature 1911 to 1959

Type of Exposure	Cases (No.)
Occupational, x-ray and radioactive materials	12
Therapeutic x-ray	
Spondylitis	55
Others	18
I^{131} therapeutic	9
Thorium	9
Atomic bomb survivors	123
Total	226

NOTE: Cases obtained from surveys, such as thymus radiation, obituary notices of United States physicians, and fetal exposure to diagnostic x-ray, were not included because of inadequate data.

leukemia per rad. Treatment with splenic or bone marrow transplants to increase the recovery rate of otherwise fatally radiated animals diminishes or reduces to 0 the yield of leukemia. The animal data suggest strongly that there is a great difference in the leukemogenic effect of radiation between whole-body and sectional radiation, indicating that the yield per rad would be different in the two cases. It is hazardous to extrapolate from animals to man but it is equally hazardous to set up assumptions for man that are in variance with a wealth of animal experimentation.

DOSE RATE DEPENDENCE

For purposes of prediction, the problem of dose rate dependence becomes critical. The single dose Japanese data are relatively good. However, the most practical situation with which one is confronted is quantitative prediction from protracted or chronic exposure over a working lifetime. There are no satisfactory data in man to demonstrate whether or not there is a dose rate dependence. Mole [*40*] in systematic studies on animal leukemogenesis has shown a definite dose rate dependence. In these experiments the dose rate was also relatively high compared to background, diagnostic and fallout radiation. Even if it were established that radiation leukemogenesis were on the basis of a somatic mutation a dose rate dependency would have to be considered since the Russells [*41*] have shown a dose rate dependency for mammalian genetic effects of radiation. However, their results are still consistent with a linear no-threshold response. These considerations indicate that predictions of leukemia incidence from acute exposure in man would greatly exceed the incidence from chronic or protracted exposure.

COMMENTS

In Table 1 are tabulated the adequately documented cases of leukemia attributed to radiation exposure. It is noted that there is only a total of 226 cases upon which all analyses are based to date. The numbers obviously are quite small. However, it appears to be established beyond reasonable doubt that large single or protracted doses are leukemogenic. Of the several sources of data considered, the only material at all suitable for a quantitative dose response relationship are the Japanese data and to a lesser extent the spondylitic data. However, these data are inadequate to establish the existence or absence of a threshold or whether the response is linear. Thus it is impossible to predict the effect of low dose levels of exposure, such as diagnostic radiation background, fallout or internally deposited radioisotopes. However, with single large doses an incidence of one to two cases per rad per year per 10^6 population at risk can be anticipated for a postexposure period of approximately fifteen years. This figure cannot be used for low doses or low dose rates.

These conclusions differ in part from those of Lewis [*6*]. We do not accept as proved the following assumptions on which Lewis based his analysis:

(1) The regression of leukemia incidence on dose is linear over a wide dose range, and down to very low dose levels (no threshold, linear hypothesis).

(2) There is no difference between the effectiveness of a single dose or the same dose protracted over a lifetime (dose rate independent).

(3) The incidence of leukemia after exposure will continue at the same rate for the rest of the life of the exposed population.

(4) There is no difference in effectiveness between whole body, thymic or spinal marrow exposure, or radiation by radioisotopes such as Sr^{89-90} deposited in the bone marrow.

(5) The dose of radiation in all situations in which there are human data on the incidence of leukemia is known or can be calculated with sufficient accuracy for prediction.

While it is generally acknowledged that the population in the United States is being subjected to an increasing level of radiation exposure, especially from medical uses of radiation, this increased exposure has not been correlated with the observed trends of the incidence of leukemia in the United States. Gilliam and Walter [*42*] have shown that between 1921 and 1955 the age-adjusted death rate for leukemia for all ages, races and for each sex has increased with a rate of increase greater in non-whites than in whites. Since 1940 the rate of increase of the age-adjusted death rate has been decreasing in the whites but not in the non-whites. These workers state, "If these trends among whites persist, they will eventually result in stabilization or actual decline in leukemia death rates for all ages. Without projection into the future, however, the trends presently evident provide no support whatsoever for a theory which postulates a sharp increase within the last fifteen years in leukemogenic factors effecting the environment of Americans in general. On the contrary, the data suggest that such exposure has become stabilized or has actually decreased during this period. If exposure to environmental factors is in fact responsible for the disease, the steady decrease in the rate of increase in leukemia mortality in every age group among the white population under seventy-five years of age is entirely consistent with the theory of recent decrease in the exposure of the general population to whatever causes operate to induce the disease." Since a strong correlation can be made between the increase in childhood leukemia between 1910 and 1950 and the use of diagnostic x-ray and thymic radiation, there is a tendency to correlate the two. It is quite clear when considering the latent period between radiation and the development of leukemia that fallout cannot be blamed for the increase between 1910 and 1940 since there was no fallout. The computed age-specific death rates are greater in the last three to four decades and an explanation is called for. Is it all due to an improved level of medical care with an increased ability for diagnosis? Certainly prior to 1930 many acute leukemias may have been diagnosed as diphtheria or other acute infectious diseases. A proportion of the increase in childhood leukemia may lie in quicker and more accurate diagnosis. However, the widespread use by the medical profession of drugs which have the capacity of producing disorders of hemopoiesis, the problem of food additives, diagnostic x-rays, smog and general industrial toxications, air and water pollution, are also factors that cannot be ignored. It seems clear that focusing of so much attention upon radiation and leukemia does not solve the broader problem and may actually be detrimental since other potentially cancerogenic agents are not being adequately studied. Whereas the data for correlation of leukemia with dose of radiation, particularly in the low dose, chronic exposure is inadequate, it is also evident that radiation is one of the most carefully studied toxic influences in our environment. No drug or toxic chemical has had its potential hazard evaluated as carefully or with as large a number of animals. The usual procedures for determination of drug and chemical toxicity probably would not detect a hazard with chances of occurring of the order of 10^{-4} per year.

Brues [*43,44*] and Lamerton [*45*] have analyzed the general problem of fitting curves for dose-effect relationship and of devising general theories of the origin of radiation-induced cancer. They have concluded that there are at the present time no *a priori* grounds for making firm statements concerning the absence or presence of a threshold dose, or for assuming any given form of relationship between the incidence of tumor and dose. Of great importance, it appears that one cannot assume that the same type of mechanism is necessarily involved in all forms of radiation-induced malignancy or leukemia. If one were to make this conclusion one might easily overestimate in some instances as well as underestimate the potential hazard in other instances. When one looks at animal leukemia (where there has been a large amount of animal experimentation) one may logically ask why the questions have not been answered. Brues [*44*], Lamerton [*45*], Kamb and Pauling [*46*] have pointed out that prohibitively large numbers of animals would be required to obtain significant experimental data at the low levels of incidence of late effects in which one is interested. There is no clearcut model for carcinogenesis from radiation at the present time but much has been learned by systematic animal studies on radiation carcinogenesis. The complexity of the situation is indicated by recent studies on radiation-induced breast neoplasia in the rat [*47–49*], indicating that probably a linear relationship holds between dose and effect over a significant range of doses, but that the breast tissue itself must be radiated,

and intact (probably cyclic) ovarian function must be present for maximal induction of neoplasia. Bond et al. [*49*] have interpreted these findings in a manner similar to that of workers in chemical carcinogenesis, namely, that there may be an "initiating" or "primary event" following exposure that may be "dormant" and not manifest itself as neoplasia unless a secondary or "promoting" stimulus is present. This would indicate strongly the great uncertainty connected with interpreting gross neoplasia incidence-dose curves in terms of primary mechanisms and indicates further the extreme uncertainty of extrapolating from one type of neoplasia to another in terms of mechanisms or predictions of amount of neoplasia, even within a single strain or species.

It has been pointed out by Brues [*43*] that the doctor or the health physicist is asked practical questions and, since progress does not or cannot wait for the scientific answers, is expected to give an answer before adequate data are present. In the case of radiation, he is forced to employ such adjectives as "safe," "permissible" or "tolerable" in order to satisfy the human, social or ethical requirements. This problem becomes extremely unscientific when one becomes involved in the area of low dose effects. In this area the theoretical scientist may take a low incidence and multiply it by the population of the world and come out with what appears to be an intolerable situation, ignoring the calculated risks in all human endeavors. When death, disease or injury is being produced by a factor in society it can only be considered in relation to the benefit obtained. Certainly, when the small toll of radiation is compared to the large positive life-saving benefits of diagnostic and therapeutic x-ray the problem is placed in a more judicious perspective. For fallout, obviously, no brief can be given since it performs no useful purpose. For industrial development of nuclear power the benefits unquestionably outweigh the small hazard. This is particularly true since fossil fuel supplies are dwindling and since the small hazard involved is avoidable by the use of protective measures. An interesting comment which helps to place the problem in humorous if not correct perspective is an article that appeared in the *London Times*, September 9, 1959. Professor Mayneord (a distinguished x-ray physicist) and associates have analyzed carefully elements of the British diet and conclude that a typical high carbohydrate British diet contains amounts of radium and thorium equivalent in effect to some 300 times the present intake of Sr^{90} from nuclear explosions. It was pointed out that 4 ounces of Brazil nuts contain three times as much radium and thorium as the entire bone and soft tissues of a normal adult. In typical British irony, "If the present level of Sr^{90} in food in this country represents a radiation hazard and it apparently does to the armchair calculator then the whole process of eating food must be regarded as highly dangerous." This is not repeated to deprecate the hazard of Sr^{90} or any increase in radiation exposure but in an endeavor to point out that the search for leukemogenic radiation factors, indeed for all harmful environmental factors, must be on a broad base, not focused upon radiation in general or the by-products of nuclear weapon testing particularly.

CONCLUSIONS

The following conclusions are made:

1. Data are now adequate to indicate that, for high level, single dose exposure of man, the incidence of leukemia is approximately linear with dose. At dose levels of perhaps 100 r equivalent or greater, the incidence is approximately 1 to $2/10^6$ persons at risk/year/rad, at least from approximately the second to the fifteenth year following exposure.
2. Below dose levels of approximately 100 r equivalent, the available data are inadequate for prediction.
3. Data are inadequate for prediction for other than the single acute dose, i.e., it is not known if a dose-rate dependency exists.
4. It is not known if the risk continues beyond approximately the first fifteen years from exposure.
5. Although there is evidence that large dose radiation of sizeable portions of the marrow may be leukemogenic, there are no adequate grounds for assuming that the highly localized radiation from internal emitters such as Sr^{90} and radium are or are not leukemogenic.
6. It is not possible to determine whether or not a threshold dose for the induction of leukemia does or does not exist.

REFERENCES

1. von Jagie, N., Schwarz, G. and von Siebenbach, L. Blutbefunde bei Roentgenologen. *Berl. klin. Wchnschr.*, 48: 1220, 1911.
2. Cronkite, E. P., Bond, V. P. and Dunham, C. L.

Some effects of ionizing radiation on human beings. A report on the Marshallese exposed to radiation from fallout. TID 5358. Washington, D. C., 1956. U. S. Government Printing Office.

3. Pathologic effects of atomic radiation. Publication 452 of the National Academy of Sciences National Research Council. Washington, D. C., 1956.
4. The hazards to man of nuclear and allied radiations. In: British Medical Research Council Report. London, England. Her Majesty's Stationery Office.
5. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. United Nations, New York, 1958.
6. Lewis, E. P. Leukemia and ionizing radiation. *Science*, 125: 965, 1957.
7. DuShane, G. (Editorial.) Loaded Dice. *Science*, 125: 963, 1957.
8. Cronkite, E. P. and Bond, V. P. Radiation Injury of Man. Its Biological, Chemical and Pathologic Basis. Springfield, Ill., 1960. Charles C Thomas.
9. Folley, H. H., Borjes, W. and Yamawaki, T. Incidence of leukemia in survivors of the atomic bomb in Hiroshima and Nagasaki. *Am. J. Med.*, 13: 311, 1952.
10. Lange, R. D., Moloney, W. C. and Yamawaki, T. Leukemia in atomic bomb survivors. I. *Blood*, 9: 574, 1954.
11. Moloney, W. C. and Lange, R. D. Leukemia in atomic bomb survivors. II. *Blood*, 9: 663, 1954.
12. Moloney, W. C. and Kastenbaum, M. A. Leukemogenic effects of ionizing radiation on atomic bomb survivors in Hiroshima City. *Science*, 121: 308, 1955.
13. Wald, N. Leukemia in Hiroshima City atomic bomb survivors. *Science*, 127: 699, 1958.
14. Heyssel, R., Brill, A. B., Woodbury, L. A., Nishimura, E. T., Ghose, T., Hoshino, T. and Yamasaki, M. Leukemia in Hiroshima atomic bomb survivors. Atomic Bomb Casualty Commission Technical Report, No. 02-59. Washington, D. C., March 1959. U. S. National Academy of Sciences National Research Council.
15. Cobb, S., Miller, M. and Wald, N. On the estimation of the incubation period in malignant disease. *J. Chronic Dis.*, 9: 385, 1959.
16. Cronkite, E. P. and Bond, V. P. Effects of radiation on mammals. *Ann. Rev. Physiol.*, 18: 483, 1956.
17. Bond, V. P. Geometrical and energy factors influencing the effect of penetrating radiation in man. In: The Shorter-Term Biological Hazards of a Fallout Field. AEC-DOD Symposium, U. S. Supt. Documents, Washington, D. C. December, 1956.
18. Court-Brown, W. M. Nuclear and allied radiations and the incidence of leukemia in man. *Brit. M. Bull.*, 14: 168, 1958.
19. Court-Brown, W. M. and Doll, R. Adult leukemia trends in mortality in relation to etiology. *Brit. M. J.*, 1: 1064, 1959.
20. Stewart, A., Webb, J., Giles, D. and Hewitt, D. Malignant disease in childhood and diagnostic irradiation in utero. *Lancet*, 2: 447, 1956.
21. Ford, D. D., Paterson, J. C. S. and Trunting, W. L. Fetal exposure to diagnostic x-rays and leukemia and other malignant disease in childhood. *J. Nat'l. Cancer Inst.*, 22: 1093, 1959.
22. Kaplan, H. S. The evaluation of the somatic and genetic hazards of the medical uses of radiation. *Am. J. Roentgenol.*, 80: 696, 1958.
23. Polhemus, D. W. and Koch, R. Leukemia and medical radiation. *Pediatrics*, 23: 453, 1959.
24. Murray, R., Heckel, P. and Hempelmann, L. H. Leukemia in children. *New England J. Med.*, in press.
25. Simpson, C. L., Hempelmann, L. H. and Fuller, L. M. Neoplasia in children treated with x-rays in infancy for thymic enlargement. *Radiology*, 64: 840, 1955.
26. Simpson, C. L. and Hempelmann, L. H. Association of tumors and roentgen ray treatment of thorax in infancy. *Cancer*, 10: 42, 1957.
27. Snegeriff, L. S. The elusiveness of neoplasia following roentgen therapy in childhood. *Radiology*, 72: 508, 1959.
28. Manning, M. D. and Carroll, B. E. Some epidemiological aspects of leukemia in children. *J. Nat'l. Cancer Inst.*, 19: 1087, 1957.
29. Conti, E. A. and Patton, G. B. Study of the thymus in 7400 consecutive newborn infants. *Am. J. Obst. & Gynec.*, 56: 885, 1948.
30. Moloney, W. C. Induction of leukemia in man by radiation. In the Symposium on Radiation Biology and Cancer. M. D. Anderson Hospital and Tumor Institute, Houston, Texas, 1959.
31. Moloney, W. C. Leukemia and exposure to x-ray, a report of six cases. *Blood*, 14: 1137, 1959.
32. Henshaw, P. S. and Hawkins, S. W. Leukemia incidence in physicians. *J. Nat'l. Cancer Inst.*, 4: 339, 1944.
33. Peller, S. and Pick, P. Leukemia and other malignancies in physicians. *Am. J. M. Sc.*, 224: 154, 1952.
34. March, H. C. Leukemia in radiologists in 20 year period. *Am. J. M. Sc.*, 220: 282, 1950.
35. Krebs, K., Rask-Nielsen, H. C. and Wagner, A. The origin of lymphosarcomatosis and its relation to other forms of leucosis in white mice. *Acta radiol.* (supp. 10), p. 1, 1930.
36. Furth, J. and Furth, O. B. Neoplastic diseases induced by general irradiation with x-rays. *Am. J. Cancer*, 28: 54, 1936.
37. Furth, J. and Upton, A. C. Leukemogenesis by ionizing radiation. *Acta radiol.* (supp. 116), p. 469, 1954.
38. Kaplan, H. S. On etiology and pathogenesis of leukemias, a review. *Cancer Res.*, 14: 535, 1954.
39. Kaplan, H. S., Brown, M. B. and Paull, J. Influence of bone marrow injection or involution and neoplasia of mouse thymus after systemic irradiation. *J. Nat'l. Cancer Inst.*, 14: 303, 1953.
40. Mole, R. H. The development of leukemia in irradiated animals. *Brit. M. Bull.*, 14: 174, 1958.
41. Russell, W. L., Russell, L. B. and Kelly, E. M. Radiation dose rate in mutation frequencies. *Science*, 128: 1546, 1958.
42. Gilliam, A. G. and Walter, W. A. Trends of mortality from leukemia in the United States, 1921–1955. U. S. Public Health Service, Public Health Report, vol. 73, p. 773, 1958.
43. Brues, A. M. Critique of the linear theory of carcinogenesis. *Science*, 128: 693, 1958.
44. Brues, A. M. Somatic effects, low-level irradiation,

p. 73. American Association for the Advancement of Science. Washington, D. C., 1960.

45. LAMERTON, L. F. An examination of the clinical and experimental data relating to the possible hazard to the individual of small doses of radiation. *Brit. J. Radiol.*, 31: 229, 1958.
46. KAMB, B. and PAULING, L. The effects of strontium 90 on mice. *Proc. Nat'l. Acad. Sc.*, 45: 54, 1959.
47. (*a*) BOND, V. P., CRONKITE, E. P., LIPPINCOTT, S. W. and SHELLABARGER, C. J. Studies on radiation induced neoplasia in the rat. III. Relation of the neoplastic response to dose of total body radiation. *Radiat. Res.*, in press.

(*b*) No. P/885, Conference on Peaceful Uses of Atomic Energy, Geneva, 1958. In: Progress in Nuclear Energy. London, 1958. Pergamon Press.

48. CRONKITE, E. P., SHELLABARGER, C. J., BOND, V. P. and LIPPINCOTT, S. W. Studies on radiation induced mammary gland neoplasia in the rat. I. The role of the ovary in the neoplastic response of breast tissue to total—or partial—x-irradiation. *Radiat. Res.*, 12: 81, 1960.
49. BOND, V. P. Radioisotope toxicity, critical review. Radioisotopes in the biosphere. In: Symposium of the University of Minnesota, Oct. 1959. To be published.

The Virus Theory of Leukemia*

JEROME T. SYVERTON, M.D. *and* JOHN D. ROSS, PH.D.
Minneapolis, Minnesota

SINCE a basis was provided by the experiments of Ellerman and Bang in 1908 [*1*], the virus theory of leukemia has been frequently debated but rarely stated. In areas of research where viral etiology once was highly hypothetical, it is now beyond question. Although presently there is no evidence for or against a viral etiology of human leukemia, even if viruses should prove minor agents of the human disease it would still be true that they are major agents of leukemia in vertebrates. With neoplasia in general, the problem now is not to justify specific biological agency but to explore the interaction of multiple etiological factors resulting in clinical disease. The significance of a virus theory of leukemia (or other form of neoplastic disease) therefore is derived from what help it may be in answering three questions: (1) does the neoplastic response ever commonly result without prior virus infection, (2) how does a virus affect a cell so as to alter growth potential rather than destroy, and (3) how can virologic measures be employed to suppress the incidence of neoplastic disease?

This review represents an attempt to assemble a picture of the role of viruses in leukemia by consideration of selected aspects of research on experimental diseases of birds and mice. Since it is already apparent that study of proliferative viruses will enlarge concepts of viral action and the spectrum of cellular response to virus infection, the picture is as much a theory of viruses as of leukemogenesis.

No exclusive definition of "virus" can yet be offered without danger of future refutation. In the laboratory, viral action is recognized by induction of effect on cell cultures or other indicators by material freed of microscopically visible microorganisms, suppression of the effect by specific antiserum, and propagation of the effect from indicator to indicator beyond the limits of dilution of the original inoculum. Concepts of the nature of viruses so recognized, as revealed by their etiological role in disease, have altered radically in the last decade. The problems of investigating oncogenic viruses should be regarded in the light of problems associated with "typical" viruses as they are now known [*2*]. With human cytopathogenic viruses, infection and disease rarely are synonymous, and lesions are uncommonly specific for the inciting virus. Under these conditions, the etiological criterion of virus isolation from a specific lesion of a specific disease has had to be replaced by the concept of epidemiological association. Frequency distributions of virus isolations, virus and antibody titers, and disease symptoms, are obtained by laboratory and clinical study of selected populations. These data provide evidence to associate prevalence and intensity of a particular virus infection with particular spectrums of symptoms. Ultimate proof of the etiological role of a particular virus depends on suppression of disease incidence by a specific viral vaccine.

Concepts of the physical nature of viruses likewise are being revised. The names "virus" and "gene" distinguish activities of macromolecular products of cells. A "pure" virus particle may be regarded as an aggregation of DNA or RNA enclosed in an envelope of protein immunologically distinct from cellular protein. This viral protein functions to facilitate penetration of viral DNA and RNA into a cell. Once within a cell, the DNA or RNA is replicated by cellular synthetic processes and re-enclosed in specific viral protein also cell-synthesized, and released again from the cell. In its purely viral manifestation, the molecular parasite is an instrument of pure reproduction; but, at least in bacteria, units of viral heredity may be incorporated into the cellular genome and behave as genes. Other findings indicate that the specificity of viral structure and function may not be absolute. Electron micrographs of some oncogenic viruses

* From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota. This study was supported in part by grants from The National Cancer Institute, and Grant No. E-42 and E-62 from The American Cancer Society.

suggest that cellular components may become associated with viral components during the process of assembly and extrusion of virus particles [*3*]. In some instances of virus infection, it seems possible that more than one viral genome may be enclosed to form a single virus particle [*4*]. The genetic components of some viruses can parasitize cells unresponsive to complete virus [*5*]. From the cellular viewpoint, perhaps virus represents a "most probable" product when viral and cellular genetic units and protein have been synthesized by cells in certain physiological states. Remaining combinations of viral protein and cellular genes, or cellular protein and viral genes, may be possible but less probable products. It is important to learn the possible associations of viral function and effect as a basis for a virus theory of leukemia. The combination of viral protein and viral genes which determines penetration and replication, and hence the infectiousness of virus, may account for viral cytopathogenic effects. May it also account for proliferative effects? Is the intracellular presence of viral genic nucleic acid in a cell in association with cellular genes sufficient for proliferative effects?

To assess the implications of a virus theory of leukemia, it is helpful to consider the possible nature of leukemia from a biological rather than a purely pathological standpoint. A virus theory need not entail a view of leukemia as a neoplastic process [*6*]. Reproduction of cells of the blood-forming series normally is devoted to (1) maintenance of parental populations in body compartments, and (2) generation of non-multiplying, functional progeny [*7*]. Abnormal concentrations of cells in blood and tissue could result from increased production of parental or of progeny cells. In the latter case leukemia could be regarded as a body response to virus infection, as leukocytosis is a body response to bacterial infection. Excessive proliferation of parental cell types, in contrast, would constitute a neoplastic process. The process would be neoplastic rather than hyperplastic if it resulted in departure of the proliferating cells from normal "compartments," either by infiltration or destructive invasion. Deleterious effects on the host could result from (1) loss of vital function through production of proliferating rather than functioning cells, (2) mechanical interference with the function of infiltrated or invaded normal tissue, and (3) competition with normal cells for vital nutrients. While effects (1) and (2) could contribute to or even determine the cause of death of leukemic patients, for research purposes effect (3) may be given major consideration, as a prerequisite to cellular invasion as opposed to infiltration. Since there are no words to distinguish malignancy as a biological phenomenon from malignancy as a pathologic process, it is of interest to consider the difference at the cellular level. The nutritional reserve of the animal body is great enough to accommodate rapid and expansive accumulation of cells in otherwise unoccupied body space, as occurs with embryonic growth. For excessive cellular proliferation to be destructively invasive in occupied body space, however, cells must possess an intrinsic capability for abnormally efficient growth as well as for evasion of growth-inhibiting influences. Disease could result from the presence of too many cells in vulnerable body tissues, or from cellular proliferation; in the first instance harm would result from interference with tissue function, in the second instance from competitive physiological activity. Malignant disease thus may include uncontrolled, and uncontrollable, growth. This distinction is the physiological implication of the differentiation of "conditioned" and "autonomous" forms [*8*] of experimental leukemia. The malignantly capable cell could be considered a genetic variant, compared to the merely proliferative cell which might be called a physiologic variant.

In this discussion we shall interpret "leukemia" noncommitively as a proliferative disease involving cells of the reticuloendothelial series. On this basis we can consider four theories of virus-induced leukemia: (1) virus infection upsets body processes governing the rate of replacement of parental cells of the blood-forming series, with resulting continuous overproduction of non-multiplying normal cells, and harm from excessive cell density in vulnerable tissue sites; (2) virus infection of the parental cells induces them to multiply excessively despite controlling body processes, with or without harm from the physiological consequences of "illegitimate" multiplication; (3) virus-conditioned multiplication of parental cells induces, or increases the probability of, emergence of an especially competent cell type that is less susceptible to influences controlling body growth; and/or (4) virus multiplication accompanying virus-conditioned cell proliferation results in a varient virus able by infection to confer special

growth competence on cells. These theories may represent alternative explanations, explanations of alternative consequences of infection by different viruses, or explanations of alternative consequences of infection of different parental cell types by one or different viruses. Two theories of virus induction of proliferative effect may be considered: (1) the effect results from the presence in the cell of virus-carried genetic material, and (2) the effect results from cellular reproduction of virus material. It may be presumed that the virus can be effective only by blocking or unblocking cellular synthesis of DNA, RNA and/or protein. Cellular response to virus and cellular reproduction of virus may be the same event, alternative events or concomitant events. Finally, possible modes of transmission of viral effect and viral infectivity may be considered: (1) virus may pass from cell to cell (or host to host) by infection resulting from cell-attachment, penetration and replication of virus; and (2) virus may pass from cell to cell (or host generation to host generation) during the course of cell division, as genetic material associated with cellular genes. Combination of possible mechanisms of virus effect with these modes of transmission of viral infectivity generates many possible modes of transmission of viral effect. Two types may be noted particularly. With respect to the epidemiological control of disease, the mode of transmission of effect rather than infectivity is important. If viral effect is transmitted *genetically* from host to host, control measures must be directed at affected *cells;* if effect is transmitted by *infection*, then control measures can be directed at the *virus*. For example, viral genes might be passed from host generation to host generation without inducing neoplastic effect until host cells in the course of multiplication converted viral genes into complete virus which, by initiation of infection, initiated leukemic response of the host. Despite genetic transmission of potential infectivity, early vaccination could prevent disease.

It is now known that an oncogenic virus can destroy cells like any cytopathogenic virus. Additionally, then, the outcome of virus-cell interaction must be considered dependent upon the physiological state of the cell at the time of contact with virus. Many or all of the theories cited and their combinations may describe possible outcomes of leukemic virus infection in different hosts of differing age, sex, environment and genetic composition. Clinicians may believe that differences between leukemia as seen in human subjects and animal leukemia as studied in the laboratory cast doubt on the validity of extrapolation of experimental findings. It is apparent, however, that if the possibilities indicated are to be explored for human populations, the methods of approach must first be learned by study of propagated, stabilized, virus strains and inbred hosts. Each model experimental system can only be considered a potential analogue of leukemia in a particular human host under particular conditions.

The complexity of the oncogenic virus problem, as herein summarized, has been analyzed similarly by Furth and Metcalf [*9*], who distinguished transmissive from causative roles of virus infection in neoplasia. The distinction is a reminder that the chief value of theorizing that viruses cause leukemia is the prospect that antiviral measures may offer promise of disease control. One can equally well conclude that poliovirus transmits but does not cause poliomyelitis, because human viral infection has less than one chance in a thousand of resulting in paralysis. Nonetheless experience has shown that control of the transmitting virus is a good approach to control of the disease. We are here considering the virus theory of leukemia as an aid to virologic control; control of radiation or other leukemogenic factors is equally desirable, whether or not they may be conceived as "prime" agents. As an object of control, Burnet [*10*] has emphasized the "central role of somatic mutation, with selective survival and proliferation of mutant clones," and considered that recent work on experimental viral leukemia "has more the flavor of a laboratory curiosity than of a realistic approach to the human problem. Homozygous strains of mice and injections of blood and tissue extracts are all equally unnatural." The even greater artificiality of non-neural tissue cultures and the cytopathogenic effect of poliovirus is poor ground for rejecting the Salk vaccine. The relative importance of associated etiological agents in natural disease is not a sure guide to control. Progressive control of microbial disease has been accomplished by interference with bacteria, the transmissive agents, rather than with the causative agents, which still are mostly unknown [*11,12*]. With leukemia and other neoplastic diseases, the virologist's task is to see how the virus theory can be put to use, and to presume

that it can be put to use so long as viruses have *any* role in leukemia.

As in human populations, leukemia occurs rarely in animal populations under natural conditions. Little experimental progress could be made until inbred animals became available for study. Most of the results discussed herein are representative of a few virus strains propagated in a few inbred lines of chickens or mice. Survey of this material, however, does not leave the impression that the restriction in species or strains should be considered to preclude generalization. An astonishingly wide range of viral, cellular and epidemiological behavior is represented. A tendency to look on the rabbit papilloma and the mouse mammary carcinoma as prototypical virus-induced mammalian neoplasms has lost justification with study of the "polyoma" virus. If unipotent virus and specific cellular response are not typically mammalian, then the complexity of the avian leukemias is not a reason for emphasizing study of mammalian over avian species.

THE AVIAN LEUKEMIAS AND ASSOCIATED CELLULAR RESPONSES

The possible complexity of the avian response to proliferative virus is illustrated by results with the RPL12 virus strain [*13*], passed by cell-free filtrate in inbred White Leghorn birds, after derivation from tumor transplants. Four responses are seen. Erythroblastosis, attributed solely to continued production of proerythroblasts in sinusoids of the bone marrow, results in invasion of the circulatory system. Proliferation of lymphoblasts in multiple foci in almost any organ produces a malignancy (lymphomatosis) in which affected foci extend by destructive invasion rather than by infiltration. Visceral lymphomatosis uncommonly involves blood invasion and frequently is accompanied by hyperplasia of erythroid elements. Osteopetrosis, a periosteal hyperplasia, and hemangiomatosis are benign conditions. In consideration of other virus strains, a general picture of the avian leukosis complex [*14*] indicates that myeloblastosis is a disease from myeloid elements, companion to erythroblastosis. The erythroblastotic vascular response may be associated with connective tissue sarcomas or renal carcinoma. Lymphomatosis may arise in neural (neurolymphomatosis) or ocular (ocular lymphomatosis) lymphoid sites. Lymphosarcoma may accompany the invasively disseminative form of response.

Assessment of the temporal distribution of mortality in birds inoculated with RPL12 virus two weeks after hatching [*15*] revealed a peak incidence of death from erythroblastosis at forty days, and from visceral lymphomatosis at 150 days. Analysis of the distributions to virus dose associated high dosage with erythroblastosis and low dosage with lymphomatosis. The age of birds at inoculation (and dose) determined the time to death from erythroblastosis, but the time to death from lymphomatosis was related rather to time after inoculation. The findings with long propagated virus were compared with results with seven strains of virus passed in inbred birds after isolation from field cases of visceral lymphomatosis. Failure to observe instances of neural or ocular lymphomatosis suggested that other viruses might be involved in these responses. Osteopetrosis was a common consequence but not when passed virus was obtained from feces, egg or other materials which might be natural sources of transmission. Hemangiomatosis was seen frequently with some passages of some of the virus lines but not all. Fibrosarcomas and myxosarcomas appeared sporadically more often in inoculated birds than in uninoculated controls.

Response incidence and composition, and incubation period, but not nature of the cellular responses, were influenced by host age and origin and by viral origin and dose. The differences between field cases of disease and the RPL12-induced disease appeared to be quantitative rather than qualitative, since responses obtained with natural sources of virus (egg extracts from naturally infected flocks, oral washings and fecal specimens) resembled those produced by diluted RPL12 virus. It was epidemiologically interesting to note that one day old chicks inoculated intravenously with dilute virus died exclusively from erythroblastosis, while uninoculated pen mates of the same age died of visceral lymphomatosis. The influence of virus dose was enough to explain effects of inoculation route, virus source and virus transmission route on incidence and variety of disease [*15*]. Analysis of incidences of virus-induced erythroblastosis and lymphomatosis in susceptible and resistant inbred lines of chickens and crossed interlines suggested that either two viruses were active or that genetic determinants of each response at the cellular level were unrelated. These findings are notable

particularly in showing how the nature of a leukemic response might be determined by the factor of virus dosage, and in providing an explanation in terms of the sensitivity of particular cell types rather than general resistance of the host to establishment of infection.

The host factors influencing response of inbred White Leghorn chickens to leukemogenic virus were resolved further by work carried out with the B.A.I. strain A virus [*16*]. This strain was derived from a case of neurolymphomatosis; original passage material induced erythroblastosis, myeloblastosis and lymphomatosis in susceptible birds, but myeloblastosis was obtained as a sole response of the strain as it now exists, following rapid passage of cell-free filtrates in young chicks. Host resistance factors were studied by inoculation of this myeloblastosis virus into birds of varying age, in varying dose by varying routes [*17*]. By relating cumulative disease incidence to the latent period preceding disease, it was possible to measure separately two types of host resistance to the development of disease. There was a broad but uniform distribution of degree of natural resistance in the inbred birds. This variation in susceptibility was independent of virus state and dose (since all birds received the same amount of filtered chick plasma as virus source), and increased with host age. Evidently each chick possessed an individually variable degree of susceptibility (or resistance), which increased with age in a manner unrelated to the initial degree. The rate of resistance increase was greater than could be attributed to a diluting effect of increased blood volume with age, but still was related to growth to the extent that the increase was greatest in rapid growth periods. With increasing time after inoculation, the birds abruptly became more resistant to the development of disease; this change was associated with the virus dose and the route of introduction. Prolonged association of birds with virus thus added active resistance to natural resistance factors. The route of virus administration was seen to determine primarily the amount of virus contributing to infection; ineffective virus did not appear to function as an antigenic stimulus.

These studies emphasized the effect of virus transmission factors on the distribution of leukemic disease in a population when the character of the disease was unaffected; RPL12 virus studies emphasized the influence of similar factors on the character of the disease. In both instances the intrinsic features of component cellular responses were unaffected by otherwise influencing factors. In combination the findings indicate a dominant role of cellular susceptibility factors in the development of disease, and suggest activity of distinct viruses. Viruses inducing erythroblastosis, myeloblastosis and lymphomatosis are antigenic in chickens [*14,18*]. The erythroblastosis and myeloblastosis viruses appear to be antigenically distinct but related [*19*]. Both viruses seem to embody normal chick protein, since both are neutralized by rabbit antiserum to normal chick tissue or protein. Myeloblastosis but not erythroblastosis virus is intimately associated also with an enzyme, adenosinetriphosphatase, and with Forssman antigen. Relation but non-identity of the viruses was indicated by the results of cross neutralization and precipitation tests. Neutralization tests also have indicated an interrelation of Rous sarcoma virus and the viruses of the three leukemic responses [*14*]. Although the distinction of specifically acting viruses is of interest to virologists, the significance of such distinction in relation to the natural role of viruses in leukemogenesis is not clear. As has been suggested with respect to myeloblastosis and erythroblastosis agents [*19*], it may be that specifically acting virus strains are variants obtained by experimental selection from a single type of virus existing under natural conditions. The question of virus distinction is important in two respects. However we classify them in research, viruses in nature do exist as heterogeneous populations of particles, each with an immunologic identity and a potentiality for etiologic activity. Study of derived laboratory strains of virus with uniform identity and potentiality need not be misleading in relation to natural pathogenesis, so long as uniformity is accomplished by selection rather than by alteration. Results with the avian leukemias do not suggest that the leukemias produced by infection of selected inbred hosts with the selected virus strains are artificial in their intrinsic nature. The question of interest to virologists concerns the second aspect of virus distinction. This question has two parts: (1) can a single cell infected with a single virus particle of immunologic identity A produce a particle of identity B, and (2) can particles all of identity A induce different types of leukemia in suitable hosts? Part (2) also is a dual enquiry: first,

whether A particles can infect, say, both proerythroblasts and promyeloblasts, and second, whether infection in each instance is equally likely to result in the particular form of cell-virus relationship necessary to oncogenic effect. With many cytopathogenic viruses cell destruction mostly is an inevitable consequence of infection, and the possibility of infection is determined by the protein coat of the virus, so that immunologic identity of the virus and its etiologic potentiality are virtually the same. The possibility of oncogenic effect introduces a basis of differentiating identity and potentiality. A major value of the avian leukemia studies is their revelation of the major element in host susceptibility as the host cell rather than the host organism. There are great differences between individual chicks and between virus strains in terms of effective virus dosage: 2×10^7 virus particles are required to produce myeloblastosis in 50 per cent of inoculated inbred chicks, compared to 2×10^5 particles to produce erythroblastosis [*14*]. These figures reflect an extremely low probability that a given virus particle in a given host can accomplish infection productive of a given leukemic response. The varying susceptibility of inbred chicks with age to induction of different leukemias also directs attention to cellular factors. Apart from any complicating influence of acquired resistance to inoculated virus, a variable low probability of host leukemic response could reflect the probability of contact between a particular virus particle and a particular cell or a particular cell in a particular state.

THE PROLIFERATIVE VIRUS-CELL INTERACTION

The host studies already reviewed indicate the importance of investigating cell-virus relationships to resolve the nature of viral oncogenesis. The necessary cell-cultural technics for assay of virus and cellular response have so far been developed and used extensively only with the Rous sarcoma virus. Although this virus is not leukemogenic, its immunologic relation to avian leukemia viruses [*14*] warrants consideration here of its relation to cells. Using tumor counts on chick chorioallantoic membrane, Rubin [*20*] showed that (1) a single virus particle could start a tumor, (2) virus of a single clone could start ectodermal or mesodermal tumors of the membrane, (3) apparently all sarcoma cells produced a small number of virus particles per generation, and (4) the ability of sarcoma cells to produce membrane tumors was associated primarily with virus production rather than with cellular invasion.

In chorioallantoic membrane studies, Prince [*21*] distinguished two phases of Rous virus production and effect: initial virus multiplication was associated with hyperplasia and subsequent necrosis of ectodermal epithelial cells, while a slower rate of virus accumulation was related to hyperplasia of mesodermal fibroblastic cells which continued with resulting sarcoma formation. This work raises the interesting possibility that the ectodermal cells could respond differently depending on the number of infecting Rous virus particles. Temin and Rubin [*22*], with monolayer cultures of chick fibroblasts, showed that a single Rous virus particle could stably alter the morphology and growth potentiality of a normal chick fibroblast so as to generate a cellular focus. The evidence indicated that monolayer foci were initiated by virus but enlarged by multiplication of altered cells. Focus formation therefore provided a quantitative assay for virus or cell alteration. The progeny of single altered cells retained their distinctive morphology and growth potential, and their ability to produce Rous virus. Alteration was not associated with any change in the number of cellular chromosomes. A highly significant finding was that, even with fibroblast populations derived from a single cell, only 10 per cent or less of the cells were susceptible to infective alteration at any given time. Transient cellular competence was implicated as a factor in this heterogeneity of susceptibility. Other studies [*23*] confirmed the earlier conclusion that all altered cells released virus and that altered cells releasing virus could divide. Other facets of the cell-virus interaction were revealed by measurement of effects of ultraviolet light and radiation on cells uninfected and infected with Rous sarcoma virus and the cytopathogenic Newcastle disease virus [*24*]. Infection of cells was measured as before by the cellular response of focus formation. The results showed that (1) ability of normal fibroblasts or virus-altered cells to divide, and ability of normal fibroblasts to initiate Rous virus production, were equally radiosensitive while the ability of altered cells to continue production of Rous virus was much more radioresistant; (2) cell alteration could not be considered a transduction, in the sense that alteration and capacity to produce virus could be transmitted independently by injection; (3) cells must be capable of multiplication to

initiate, but not to continue, virus replication; and (4) the capacity of the fibroblasts to initiate production of the cytopathogenic Newcastle disease virus was much more radioresistant than their capacity to divide. Investigation of early stages of the Rous virus infection of chick embryo cells in culture [*25*] indicated only quantitative differences from other cell-virus relationships, except for the unique quality of the capacity to initiate virus production. Radioresistance of cellular capacity to continue virus production appeared in from five to twenty hours after infection; the time for development appeared shorter for higher initial multiplicity of infection.

In relation to the present discussion, the major implications of these studies with an *in vitro* system are the demonstration of cellular alteration as a direct consequence of virus infection, and demonstration of the genetic transmission of cellular alteration and virus infection. In this simplified system, infection without alteration and alteration without continued infection do not appear to be significant alternatives to the association of infection and alteration. Transient susceptibility of the individual cell, and the intimate association between cellular abilities for division and initiation of virus production, suggest that cell alteration and infection are dependent on cellular reception of virus at a particular stage in the process of replication of cellular components. Although both alteration and infectivity are transmitted genetically from cell to daughter cell, the fact that cellular capacity to produce virus can be retained when capacity for multiplication is lost leaves the status of the cellular alteration undetermined. Is the cellular alteration virus-initiated only, or virus-driven? Experimentally the question is equivalent to asking whether alteration and virus-production capacity can be segregated genetically. The possibility of such segregation, if the two properties are separate, presumably would depend on the frequency of mitotic recombination in cells multiplying in culture. In view of the earlier remarks on the significance of genetic transmission of viral effect and viral infectivity, it may be noted that these studies with the Rous virus do not necessarily imply genetic transmission of effect in the sense noted previously. Transmission in the earlier sense would imply appearance of altered cells among the progeny of an unaltered infected cell after many generations, independent of release of infectious virus. The Rous virus-chick fibroblast system thus far may be regarded as a model of virus-induced cellular alteration as opposed to destruction. It is additionally interesting that an indisputable virus infection should produce what could be interpreted as a somatic mutation [*23*].

THE MURINE LEUKEMIAS

Induction of lymphoid leukemia and/or parotid gland tumors in inbred mice by cell-free filtrate of tissue from mouse strains with a high incidence of "spontaneous" leukemia has been well documented [*26–33*]. The lymphoid cellular response in mice appears similar to the response in chickens, allowing for anatomical and physiological differences. As with birds, the murine response is dose, host-strain and host-age dependent, with respect to both incidence and composition. Strain and age differences in host susceptibility to induction of lymphoid leukemia and parotid gland tumors by cell-free filtrates of the same tissue source again raises the questions of distinct viruses already given consideration in relation to avian leukemias. Gross [*33*] showed that cells of lymphoid leukemia, parotid gland carcinoma or fibrosarcoma transplanted within the strain of origin reproduced the specific neoplastic response, while cell-free extracts in susceptible mice transmitted either leukemia, carcinoma or sarcoma, to emphasize again the cellular origin of the cellular response. Again the question of distinctness of viral infectivity in contrast to viral effect is of interest and without answer. In this respect all the virologic investigation of neoplasia to date has been uninformative: study of murine leukemias and associated neoplasms likewise does not indicate whether cells of a single cytologic origin can manifest various degrees of alteration (other than cytopathogenic effect) in response to infection by one or various viruses. The Gross strain A lymphoid leukemia virus [*33*] will infect older mice of a susceptible strain more readily than will natural source virus, to produce an earlier and higher incidence, but the character of the lymphoid response appears the same. Without more information, it can be assumed only that enhancement results from greater dosage of the derived virus. While one may be tempted by the complexities of experimental viral leukemia to look on virology as an aid to understanding the neoplastic process rather

than as an approach to control of human leukemia, the impressive virological accomplishments of recent years mostly support the reverse contention. Studies to relate pathological criteria to biological bases are badly needed to reveal the relationship between leukemogenic virus infection and the development of malignancy.

The role of host factors (that is, of the target cell environment) in the development of murine lymphoid leukemia is shown by the effects of hormones, radiation and thymectomy on incidence [*34*]. In some but not all high incidence mouse strains, leukemic cells appear to come from the thymus; with both strain types thymectomy depresses incidence. The incidence may be increased by radiation unless accompanied by thymectomy or partial protection of bone marrow or spleen with lead shields. Leukemogenesis in these experimental circumstances is dependent on treatment of the host, not the thymus. The neoplastic process appears to be enhanced or inhibited by hormones that might be expected to enhance or inhibit lymphocytic processes, but not necessarily independently of other effects. Kaplan [*34*] offered the hypothesis that thymic injury from one cause or another led to an increased proliferative stimulus to which the thymus could respond normally only if a bone marrow factor was available: without the factor, application of the stimulus resulted in the development of leukemia. Gross [*35*], with his virus indicator, low natural incidence C3H mice, showed that thymectomy blocked the development of leukemia from his potent A virus when performed before or up to a month after inoculation. Leukemia produced by injection of cells was unaffected by thymectomy. Fractional total body radiation of mice with a low natural incidence of lymphoid leukemia induced a high incidence, but did not affect the natural incidence in Ak mice (a high natural incidence strain) [*36*]. Indicator C3H mice inoculated with virus at birth and radiated when adult exhibited only a moderate increase in incidence in comparison with radiated inoculated control mice. With these C3H mice, cell-free extracts of leukemic material from radiated donors produced about a twentyfold greater incidence of leukemia than did extracts from unradiated donors after injection into newborn mice [*37*]. The incidences of parotid gland carcinoma, in contrast, were similar. Cell-free filtrate of one radiation-induced leukemia was used to propagate a virus strain by serial passage in suckling mice: neutralization tests with antiserums to this and strain A virus suggested a relation, although not conclusively [*38*]. Lieberman and Kaplan [*39*] reported lymphoma induction with filtrates of radiation-induced leukemia and lymphoma, and a lymphoma which had been propagated for seven years by transplantation. Filtrates prepared from thymus at various times after radiation were not active before sixty-four days and were more active after 128 days. The incidences of leukemia in mice given injections or not given injections with filtrate when newborn, and treated with x-rays two, six, fourteen or thirty days after birth, were similar with one exception: the mice given injections and x-ray treatment at thirty days showed a greater response. In relation to these findings it is of interest that the lymphoid leukemia virus apparently can pass from generation to generation in infectious form without necessarily inducing leukemia [*26,33*]. While strictly speaking these observations only show recovery of injectibly effective material, the extremely low infective efficiency even of highly concentrated viral ribonucleic acid (genetic material) [*40*] supports the tentative concept of *infectious* rather than genetic transmission of the infectivity of lymphoid leukemia virus. Avian and murine lymphoid leukemias appear to be egg-borne diseases [*33*], but this property does not imply genetic transmission of infectivity in the sense used here.

Miller [*41*] thymectomized four week old mice inoculated or not inoculated at birth with strain A Gross leukemia virus. Uninoculated mice received subcutaneous grafts of thymus from day old, uninoculated mice of the same strain from one to thirty days after thymectomy; inoculated mice similarly received whole thymuses up to six months after thymectomy. Subsequent leukemia was associated with reception of virus, not thymus. This observation suggests that the thymus is not simply a site of virus storage. Assuming infectious congenital reception of virus, one may assume that some non-thymic cells must be susceptible to virus infection; then, as Miller [*41*] notes, the thymus may be a source of humoral factor in leukemogenesis, the only source of a cell type susceptible to viral alteration, or more probably the only concentration of susceptible proliferating cells responsive to virus infection with resulting cellular alteration. The next problem is to connect viral and thymic influence with radiation effect in the induction

of lymphoid leukemia in low incidence mice. Lieberman and Kaplan [*39*] noted in this relation the action of radiation in the *induction* of temperate bacterial viruses (conversion of cell-carried viral genes into complete infectious virus), but such an inducing effect would imply genetic transmission of viral infectivity. Apart from any evidence against such transmission of murine lymphoid leukemia, this role of radiation is not apparently consistent with a protective effect of minor bone marrow shielding unless the shielded cells are a source of virus. Kaplan's original hypothesis of leukemogenesis [*34*] does not seem irreconcilable with viral etiology: if thymic cells were normally dependent on bone marrow factor for proliferation, and if independence of the factor were the alterative consequence of virus infection, then the influence of hormones and radiation would be understandable since the altered cells would not be competitive with normal cells in growth until the deciding factor was depleted. The manifestation of altered cells would be inhibited or enhanced by hormonal inhibition or stimulation of thymic cell proliferation. There is no evidence to support this suggestion over any other: radiation also could suppress host resistance to virus infection and hence enhance the spread of virus passage from one cell to another more likely to respond to infection by proliferation.

Continued study of the induction of virus-transmitted leukemia in low incidence mouse strains should be valuable in permitting assessment of the role of hormones, radiation and other factors in leukemogenesis. The nature of inbred host strains, however, must be kept in mind. By itself, inbreeding does not eliminate host variation, but only ensures that the extent of variation between litters is not greater than within litters. The specific character of an inbred strain is imparted by selection, commonly for or against high incidence of a particular neoplastic disease. Deliberate selection always is accompanied by selection for other characters, known or unknown. Each experimental inbred *strain*, not host, therefore should be looked on as an example of a particular response to viral and other etiological factors in leukemogenesis. The major determinant with some mouse strains may be virus, with others radiation or other factors: it can be considered only that each strain exaggerates some factors over others usefully.

In relation to human populations, the naturalness of the lymphoid leukemia of high incidence mouse strains, compared to some other experimental mouse leukemias now being studied, appears irrelevant. Three other varieties of murine leukemia have been developed experimentally by passage of cell-free filtrates in suitable mice. Friend [*42*] and Graffi [*43*] derived leukemias in Swiss and inbred Agnes-Bluhm mice, respectively, with filtrates from the long-transplanted Ehrlich ascites carcinoma of mice. The leukemias were myelogenous and unusual to the extent that inbred mouse strains with a high incidence of similar leukemia have not been described. With serial passage of filtrates, Friend derived a virus [*44*] inducing the myeloid response in adult Swiss (and inbred DBA/2) mice within three weeks or less after inoculation. The virus stimulates production of neutralizing antibody in mice and rabbits, is not related immunologically to other murine leukemic viruses, and is susceptible to control by active or passive immunization of the Swiss host mice. Infected mice do not transmit virus by contact. The cellular response is revealed by massive infiltration of the liver, spleen and bone marrow with multiplying mononuclear cells, presumably of reticular origin. Inoculated mice die two to three months after inoculation. The sequence of blood changes includes lymphopenia seen the day after virus injection, lymphocytosis and erythroblastosis, and appearance of monoblastic cells making up about 10 per cent of the white cell population [*45*]. Adrenalectomy and thymectomy are ineffective, and splenectomy merely increases hepatic enlargement. Apparently cell injection results in virus passage, because local tumors are not produced intramuscularly or subcutaneously [*45*], and antiserum is as effective against cells as against filtrates [*44*]. Since the Swiss mice are heterozygous, it can be concluded that the cells if malignant are not autonomous enough to prevail over homograft barriers. Antibody neutralization of cellular transmission of the leukemia is interesting in suggesting that the cells may not carry or release infectious virus (depending upon whether antiserum treated, washed cells also are ineffective). Whatever the degree of cellular malignancy, the disease appears to be neoplastic [*45*] and the virus thus a leukemogenic virus obtained from ascites carcinoma cells.

How difficult is the problem of oncogenic viral origin, already evident with the Friend virus, is pointed out by the studies of Schwartz and associates [*6*]. Brain filtrates from leukemic mice

of the high lymphoid incidence AKR strain, on inoculation into young AKR mice, induced more and earlier leukemia than was normal for the strain. Filtrates of non-leukemic AKR brain, AKR lymphocytic tumor and non-leukemic Swiss mouse brain, and heated filtrates of leukemic AKR brain, were inactive. These leukemias were typically lymphoid. Filtrates of brain from human patients dying of lymphoblastic or myeloblastic leukemia, but not normal brain, similarly were active in accelerating lymphoid leukemia in AKR mice. Transmission of brain filtrate also was demonstrable in Swiss mice with lymphoblastoma. This tumor, originally found in a Swiss female caged with an AKR male, was transplantable to Swiss or inbred dba mice but not to AKR mice, and therefore was presumably of Swiss origin. Leukemic Swiss brain filtrates induced lymphoblastoma in Swiss mice, appearing shortly after inoculation and reaching high incidence at the end of a month. Such tumors were rare in Swiss and dba mice, even in those a year old. Activity of leukemic brain filtrates of AKR and Swiss mice and of human origin was maintained through five rapid serial passages by filtrate in heterologous mice (Swiss for AKR and human source material, C3Heb for Swiss source material), as determined by injection of mice used to show original activity. Similar passage of non-leukemic or heated material produced no activity. All the data appear adequate to support the idea of viral agency, except for the difficulty of origin. If human brain as a source of virus active in mice is questionable, then the passage data are questionable; if the agent of the Swiss lymphoblastoma is considered a virus of Swiss or AKR origin, then a human virus can be considered the source of the human leukemic brain activity. No arrangement of controls seems sufficient for proof of viral origin; convincing evidence requires the presence of neutralizing antibody in the host(s) of presumed origin. Maintenance of activity through serial passage dilution is one part of the demonstration of viral action; the other part is neutralization of the final activity by antibody to the original material. This consideration is pertinent to virologic study of human leukemia, where the host of origin will not be available on any experimentally useful scale. Either antibody must be obtained from the virus-yielding host, or the virus must be propagated and identified with human cell cultures before introduction into animals. Since mice infected with the virus of mammary carcinoma do not demonstrably produce neutralizing antibody, antibody production is not a necessary accompaniment of oncogenic virus infection. It may also be that failure to produce antibody is one of the characters bred into a mouse strain that permits expression of virus infection in high incidence of a neoplasm of a particular type.

Potency of brain compared to tumor tissue filtrate in passage of the Swiss lymphoblastoma has been associated with activity of heat labile, low molecular weight inhibitor present in the tumor tissue [*46*]. Greater infective activity of brain extract with intraperitoneal inoculation than with intracerebral inoculation, together with evidence for tumor tissue inhibitory activity, makes the results with the Swiss virus and Gross' Ak virus [*33*] less contradictory, and indicates another factor to complicate the etiological study of leukemia.

Like the Rous sarcoma virus, the polyoma virus studied by Stewart and Eddy [*47*] has model aspects that make it worth considering here, although it is not specifically leukemogenic. The induction of parotid gland carcinoma and other tumors, as well as lymphoid leukemia by inoculation of filtrates into newborn mice, was discussed earlier. Extracts of parotid gland tumors induced by filtrate in hybrid mice were not active by direct mouse inoculation, but produced multiple tumors in mice of the same cross (but not other types) after passage in cell cultures of monkey kidney [*48*]. Similar recovery of multipotent viral activity has been accomplished by use of mouse-embryo monolayer cultures, cultures of adult mouse kidney and bone marrow [*49*], and cultures of an established strain of carcinogen-induced malignant mouse lymphoma cells, the P388D strain [*50*]. Mouse cell cultures exhibit a cytopathogenic effect [*51*] which can be used for quantitation of viral infectivity by plaque assay [*52,53*]. The virus is extraordinary in causing tumors of glandular and skin epithelium, connective tissue and endothelium in mice [*54*], invasive renal angiosarcomas in rats [*55*], sarcomas and angiomas in hamsters [*56*] and subcutaneous fibromatoses in rabbits [*57*]. Mice are susceptible to the virus as newborns as they are to direct filtrate induction of parotid gland tumors [*33*], while hamsters do not show such age limitation of susceptibility. Some inoculated mice show non-neoplastic effects, including dwarfism, anemia, acute

inflammation of the eye and glomerular nephritis [*54*]. Thymic lymphomas as well as other tumors and growth-stunting have been observed in Swiss mice inoculated when newborn with material passed in mouse cell cultures; the material, derived from mammary gland tumor tissue of a C3H mouse, was cytopathogenic for C3H and Swiss mouse kidney cultures but not for monkey or rabbit kidney cultures [*58*]. The polyoma virus is highly stable in extracellular environments [*59,60*], is antigenic and unrelated immunologically to Friend virus and a variety of other known cytopathogenic viruses [*47,61*], and is hemagglutinative [*62,63*]. This last property facilitates epidemiologic study by permitting use of the hemagglutination-inhibition test for titration of antibody, and of antibody production in weanling mice for detection of virus [*64*]. Rabbit antibody both inhibits hemagglutination and neutralizes infectivity [*47*]. Although polyoma and myxoviruses are unrelated in size, stability and immunological activity, apparently the same erythrocyte receptors are involved in hemagglutination by polyoma and myxoviruses [*65*]. If tissue cell receptors also are identical, the uniqueness of the cellular response to this oncogenic virus can be dissociated from the mechanism of its reception. Some evidence suggests that the viral particle rather than a soluble antigen is the unit of hemagglutination [*66*]. Plaque assay of virus obtained by cell culture passage from virus-induced mouse parotid gland carcinoma, mammary adenoma and kidney sarcoma has shown that one particle can be infective, that infected cells can be enumerated by their ability to generate plaques, and that virus isolated from a single plaque can produce tumors in mice and hamsters [*52*]. Polyoma virus, isolated from the spleen of an Ak mouse with lymphoid leukemia by observation of the cytopathogenic effect in mouse embryo cell cultures, has been found to produce acute hemorrhagic disease as well as sarcomas on inoculation into young hamsters, while producing virus-yielding kidney sarcomas and mammary gland tumors in mice [*67*], to suggest further that oncogenicity is a function of cellular and host factors as well as virus. The murine cellular response to polyoma virus *in vivo* is complex and unlike that seen with naturally occurring murine neoplasms; polyoma-induced tumors of various epithelial types seem to represent an *in situ* hyperplasia of cells of relatively normal morphology, combined with rapid and continuous compressive proliferation of undifferentiated cells of uncertain origin [*68*]. This suggestion of quasi-malignancy is reinforced by the partial success of transplantation of induced inbred mouse tumors in preconditioned and newborn mice of the same strain [*69*]. Since the theme of associated cell destruction, hyperplasia and neoplasia recurs in studies of avian leukemias, Rous sarcoma (the discrepancy in Prince's and Rubin's findings on ectodermal response is notable) and the response of more than one species to polyoma virus, it is reasonable to regard the peculiarities of polyoma infection as exaggerations of responses less evident in the development of other neoplasms, rather than as unique effects. Complexity also is seen in cell-virus relations. Serial transplantation in adult hamsters of cells of fibrosarcoma induced by polyoma virus in a suckling hamster only occasionally was accompanied by antibody production [*70*]. Since high levels of hemagglutination-inhibiting antibody in immunized hamsters did not prohibit tumor development from implanted cells, presumably cellular propagation was occurring. Tissue culture passage of the transplanted tumor yielded no virus. In other studies [*67*], extracts of hamster tumor were infectious for cell cultures but not for newborn or adult hamsters unless first passed in tissue cultures; cultures of the transplantable tumor yielded both virus and cells causing tumors in hamsters. Cultural studies of the ability of virus-induced tumor cells to produce and release virus showed extremely wide variations within and with tumor type, and with host species [*71*]. That actively acquired host immunity has an influence was suggested by the observation that Swiss mice inoculated with virus *in utero* died earlier from a higher incidence of tumors than did mice inoculated when newborn [*68*]. Injection of virus into mice of mothers exposed to virus by contact resulted in fewer salivary gland and renal tumors. A protective effect was shown by immunization of hamsters to challenge inoculation by use of serum neutralized virus as vaccine (formalinization proving insufficient for virus inactivation) [*72*]. Since exposure of suitable hosts to polyoma virus can elicit responses ranging from acute infection to renal hyperplasia to metastasizing osteogenic sarcoma, the role of virus in the development of malignancy (as opposed to its more general role in neoplasia) is uncertain. Sponge fragment cultures of newborn mouse salivary gland tissue

exposed to culture propagated parotid tumor (polyoma) virus have been observed to exhibit acinar proliferation resembling that seen during *in vivo* development of parotid tumors [*73*] but neither the cells in the sponge fragments nor those established on the culture vessel walls produced tumors at the site of implantation into preradiated mice of the same strain. Tumors did appear away from the site of implantation with virus exposed cultures but not with controls, presumably from transmission of surviving or propagated virus. Cytochemical and electron-microscopic evidence suggests that the cellular course of virus infection begins in the nucleus and spreads to the cytoplasm [*67,74*], as might be expected of a DNA virus. The results of epidemiologic studies made possible by the hemagglutination test are instructive [*61*]. Studies of various mouse stocks have indicated that: (1) although many mouse stocks have had experience of the polyoma virus (apparently often by inadvertent experimental contamination), some stocks with a high natural incidence of leukemia or mammary carcinoma have not; (2) mice with naturally occurring leukemia may show no antibody to polyoma virus, while mice with inoculation-induced parotid gland tumors or lymphoid leukemia generally do have antibody; and (3) mice of one C3H stock showed no antibody unless bearing the C3H leukemia described by Schoolman and associate [*6*]. No antibody to polyoma virus was found in the serum of human subjects, healthy or afflicted with solid tumors or leukemia [*61*]. Some work suggests that mice of heavily infected stocks acquire virus after birth [*64*], and that mice in infancy may be protected from infection by antibody passively transferred from mothers across the placenta or in milk [*75*]. Mice appear susceptible to infection by inhalation [*61*] or contact, and virus can be spread by mouth or excreta [*76,77*]. The evidence shows that polyoma virus is not a relative of the Gross lymphoid leukemia virus (as Gross' results also suggested), and that its protein constitution has not necessarily been altered by the experimental manipulation involved in its discovery. Its lack of relation to Gross virus shows that its isolation from leukemic tissue (or the isolation of other oncogenic viruses from tumor tissue) is not evidence of etiologic activity. Apparently, tumors harbor passenger tumor viruses as readily as other viruses, and the difficulties of etiological indictment encountered with enteroviruses may be anticipated with human oncogenic viruses.

Consideration of the murine polyoma virus completes a general picture of experimental leukemogenesis and associated neoplasia. Any fear that a virus theory is too simple an explanation of the development of neoplasia seems groundless. No specific theory of virus oncogenic action is yet suggested by the experimental results available but a general framework can be conceived for theory construction. This general concept would imply that virus infection can result in cellular destruction or not; if not, one of the consequences can be hyperplastic growth, depending on the species, type and physiological state of the infected cell; the sequence of events connecting virus reception, virus infection and cellular response may be determined by host or environmental factors affecting infection or response. The development of experimental methods for controlled manipulation of host and virus is well in hand; the vital need is for cultural technics for the quantitative assessment of cellular response. Without knowledge of the cell-alterative effects of virus, it is impossible to understand how virus infection results in malignancy (given the opportunity), or to see why *in vitro* infection by polyoma virus causes cell destruction rather than proliferation when Rous sarcoma virus does the reverse. Before virus-induced neoplasia can be understood in terms of cellular enzymes and metabolism, there is need for measurement in terms of cell growth capacity and its relation to nutritional elements and activity of other cells in controlled cultural environments. Fortunately for the problem of controlling the incidence of human leukemia, the experimental models considered here indicate that methods for isolation, identification and titration of virus and antibody are of greatest importance to the assessment of viral leukemogenesis, apart from understanding.

In human leukemia we have both lymphogenous and myelogenous conditions, with peak respective incidences in early childhood, youth and advancing years, and an over-all mortality rate of approximately 10 per 100,000 [*78–81*]. Since experimentally we would consider a hundredfold greater incidence satisfactory evidence of the lack of response of negative controls, there seems little justification for detailed comparisons of any type of human leukemia with the response of an inbred animal strain to any of the leukemogenic viruses. Neither similarities nor

differences could be interpreted as supporting the possibility of viral etiology. Greater differences between human and animal leukemias than appear to exist would not exceed observed differences within viral leukemogenesis as it is now known. The experimental models point out method, and it can be seen that the method is approaching the stage of adequacy for direct investigation of human leukemia.

The operational consequences of a virus theory of leukemia are well illustrated in studies of the polyoma virus. These consequences are worth emphasizing as approaches to the virologic study of human diseases. The first objective is the isolation of human tumorigenic viruses; for this purpose it is necessary (1) to isolate and titrate unknown viruses, (2) to show proliferative action, and (3) to establish human origin. When "proliferative human orphan" (PHO) viruses and antibody to them can be identified, the next objective will be to establish an etiological relationship between these viruses and human leukemia or other neoplastic diseases. Accomplishment of this objective will require epidemiological analysis of the qualitative and quantitative distribution of virus infections in human populations of different ages and backgrounds, for comparison with the distribution of leukemias. With other orphan viruses, the association of infection and disease has depended largely on epidemic occurrence of infections and symptoms; that is, the implicating finding has been change in incidence, rather than incidence itself. Characterization of animal leukemogenic viruses also has depended heavily on provision of epidemic conditions for study. Epidemic distribution of virus-induced neoplasia in experimental animals has been realized by use of inbred populations. With human leukemia, therefore, it may be that types of disease most useful for virologic characterization are those for which radiation or chemical agents appear to be the prime causes. In planning research on leukemia it is desirable to entertain the broadest rather than the most limited virus theory. The broadest theory would look on virus-conditioned cell proliferation as resulting in emergence of genetically variant, physiologically competitive cell clones no longer dependent on virus for their malignancy. These cells could still carry the leukemogenic virus in one form or another. The derivation of the Friend virus, for example, is consistent with this possibility. Assay of human leukemic material perhaps should be looked upon conservatively as a probable means for isolation of human leukemogenic viruses, but not etiological indictment. Short of vaccinal suppression of incidence, human antibody response may provide the most productive epidemiological evidence of etiology. It is not yet apparent which of the avian or mammalian leukemias should be regarded as an appropriate model for prediction, to the extent that epidemiologists should expect to look for increased or decreased antibody response as evidence of human viral guilt. An answer is provided, however, by another consideration. It was pointed out earlier that induction of neoplastic effect by leukemogenic virus must depend on infectious as opposed to genetic transmission if virologic control is to be practicable. Likewise, if virologic approaches to epidemiological characterization of human leukemia are to be successful, induction must *involve* infection even if proliferative effect results from the presence rather than the replication of viral genes. If there is no apparent relation between disease and infection, a virus theory of etiology has little significance, however true. With orphan viruses, increased antibody response is an unquestionably necessary indicator of virus infection. With virus-induced neoplasia, infection precedes disease by a considerable interval; even if a decreased response is found when disease is evident, an increased response may be observable earlier. Some human patients may be truly unresponsive immunologically to an existing leukemogenic virus; the virologist must hope that they do not constitute a large proportion of leukemic populations.

These considerations indicate the extreme importance of cell cultural procedures for the investigation of viral infection in leukemogenesis. Any observed abnormal effect of human source material must be associated with virus, with human virus, and with human proliferative virus, by reasonably convenient technic before epidemiological studies can be undertaken. Ironically, while cell cultures have been developed mainly for studies of viruses that do not reproduce diseases in readily available laboratory animals, the fact that analogous neoplastic diseases do occur in laboratory animals is a good reason for preferring cell cultures for study of the human proliferative viruses. Whatever the danger of activating indigenous viruses by injection of human material, there are only two ways to discount the possibility when the danger has

been introduced by use of non-human indicators: (1) by neutralizing infectivity with antiserum prepared to virus propagated in human cells, and (2) by failing to neutralize infectivity with antiserum to known proliferative viruses of animal origin. Even with human cell cultures, the necessity remains to identify the test material rather than the indicator as the source of isolated virus. It is desirable but not so acutely to use cell cultures for demonstration of the proliferative as well as the viral and human nature of leukemogenic agents. While it may be premature to consider a proliferative effect, in the sense of Rubin's "focus-forming effect," an essential property for indictment of a human virus as oncogenic or leukemogenic, it would be a helpful property to aid elimination of unknown non-oncogenic viruses.

The last question is what should be accepted as sufficient evidence of a viral etiology of human or other leukemia. The answer is a conclusion of the virus theory of leukemia, and also its major goal: suppression of incidence by vaccination. Burnet [*10*] has recommended acute childhood leukemia as the most hopeful object for epidemiological investigation of the human problem. Implications of the virus theory as reviewed herein support the recommendation of this aspect of the problem for initial testing of the theory. This form of human leukemia is all the more recommended because it should be possible to obtain a decision within five years after test. If this incidence can be suppressed by virologic control, questions of the status of virus as etiological agents and of the role of viruses in other human leukemias can be attacked from the strength of experience instead of theory.

REFERENCES

1. Ellerman, V. and Bang, O. Experimentelle Leukämie bei Huhnern. *Zentralbl. Bakt.*, 46: 595, 1908.
2. Huebner, R. J. The virologist's dilemma. *Ann. N. Y. Acad. Sc.*, 67: 430, 1957.
3. Bernhard, W. Electron microscopy of tumor cells and tumor viruses. A review. *Cancer Res.*, 18: 491, 1958.
4. Granoff, A. Studies on mixed infection with Newcastle disease virus. II. The occurrence of Newcastle disease virus heterozygotes and study of phenotypic mixing involving serotype and thermal stability. *Virology*, 9: 649, 1959.
5. Holland, J. J., McLaren, L. C. and Syverton, J. T. The mammalian cell virus relationship. IV. Infection of naturally insusceptible cells with enterovirus ribonucleic acid. *J. Exper. Med.*, 110: 65, 1959.
6. Schwartz, S. O. and Schoolman, H. M. The etiology of leukemia: the status of the virus as causative agent—a review. *Blood*, 14: 279, 1959.
7. Leblond, C. P. and Walker, B. E. Renewal of cell populations. *Physiol. Rev.*, 36: 255, 1956.
8. Furth, J. Conditioned and autonomous neoplasms: a review. *Cancer Res.*, 13: 477, 1953.
9. Furth, J. and Metcalf, D. An appraisal of tumor-virus problems. *J. Chronic Dis.*, 8: 88, 1958.
10. Burnet, F. M. Leukemia as a problem in preventive medicine. *New England J. Med.*, 259: 423, 1958.
11. Freeman, V. J. Studies on the virulence of bacteriophage-infected strains of Corynebacterium diphtheriae. *J. Bact.*, 61: 675, 1951.
12. Dubos, R. J. Biochemical Determinants of Microbial Diseases. Cambridge, Mass., 1954. Harvard University Press.
13. Gross, M. A., Burmester, B. R. and Walter, W. G. Pathogenicity of a viral strain (RPL12) causing avian visceral lymphomatosis and related neoplasms. I. Nature of the lesions. *J. Nat. Cancer Inst.*, 22: 83, 1959.
14. Beard, J. W. Viruses as a cause of cancer. *Am. Scientist*, 46: 226, 1958.
15. Burmester, B. R., Gross, M. A., Walter, W. G. and Fontes, A. K. Pathogenicity of a viral strain (RPL12) causing avian visceral lymphomatosis and related neoplasms. II. Host-virus interrelations affecting response. *J. Nat. Cancer Inst.*, 22: 103, 1959.
16. Eckert, E. A., Sharp, D. G., Beard, D., Green, I. Beard, J. W. Virus of avian erythromyeloblastic leukosis. IX. Antigenic constitution and immunologic characterization. *J. Nat. Cancer Inst.*, 16: 593, 1955.
17. Eckert, E. A., Beard, D. and Beard, J. W. Dose-response relations in experimental transmission of avian erythromyeloblastic leukosis. V. Influence of host age and route of virus inoculation. *J. Nat. Cancer Inst.*, 15: 1195, 1955.
18. Burmester, B. R. *In vitro* and *in vivo* neutralization of the virus of visceral lymphomatosis. *Proc. Soc. Exper. Biol. & Med.*, 90: 284, 1955.
19. Beard, D., Beaudreau, G. S., Bonar, R. A., Sharp, D. G. and Beard, J. W. Virus of avian erythroblastosis. III. Antigenic constitution and relation to the agent of avian myeloblastosis. *J. Nat. Cancer Inst.*, 18: 231, 1957.
20. Rubin, H. Quantitative relations between causative virus and cell in the Rous no. 1 chicken sarcoma. *Virology*, 1: 445, 1955.
21. Prince, A. M. Quantitative studies on Rous sarcoma virus. III. Virus multiplication and cellular response following infection of the chorioallantoic membrane of the chick embryo. *Virology*, 5: 435, 1958.
22. Temin, H. M. and Rubin, H. Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. *Virology*, 6: 669, 1958.
23. Rubin, H. and Temin, H. M. Infection with the Rous sarcoma virus *in vitro*. *Fed. Proc.*, 17: 994, 1958.
24. Rubin, H. and Temin, H. M. A radiological study of cell-virus interaction in the Rous sarcoma. *Virology*, 7: 75, 1959.
25. Temin, H. M. and Rubin, H. A kinetic study of infection of chick embryo cells *in vitro* by Rous sarcoma virus. *Virology*, 8: 209, 1959.

26. Gross, L. Transmission of Ak leukemia into newborn mice of the C57 Brown/cd inbred line. *Proc. Soc. Exper. Biol. & Med.*, 86: 734, 1954.
27. Law, L. W., Dunn, T. B. and Boyle, P. J. Neoplasms in the C3H strain and in F_1 hybrid mice of two crosses following introduction of extracts and filtrates of leukemic tissues. *J. Nat. Cancer Inst.*, 16: 495, 1955.
28. Woolley, G. W. and Small, M. C. Experiments on cell-free transmission of mouse leukemia. *Cancer*, 9: 1102, 1956.
29. Furth, J., Buffett, R. F., Banasiewicz-Rodriguez, M. and Upton, A. C. Character of agent inducing leukemia in newborn mice. *Proc. Soc. Exper. Biol. & Med.*, 93: 165, 1956.
30. Dulaney, A. D. Parotid gland tumor in AKR mice inoculated when newborn with cell-free AK leukemic extracts. *Cancer Res.*, 16: 877, 1956.
31. Gross, L. Development and serial cell-free passage of a highly potent strain of mouse leukemia virus. *Proc. Soc. Exper. Biol. & Med.*, 94: 767, 1957.
32. Gross, L. High susceptibility of 1 to 14 days old C3H mice to "passage A" leukemic filtrates. *Proc. Soc. Exper. Biol. & Med.*, 97: 300, 1958.
33. Gross, L. Viral etiology of "spontaneous" mouse leukemia: a review. *Cancer Res.*, 18: 371, 1958.
34. Kaplan, H. S. On the etiology and pathogenesis of the leukemias: a review. *Cancer Res.*, 14: 535, 1954.
35. Gross, L. Effect of thymectomy on development of leukemia in C3H mice inoculated with leukemic "passage" virus. *Proc. Soc. Exper. Biol. & Med.*, 100: 325, 1959.
36. Gross, L., Roswit, B., Mada, E. R., Dreyfuss, Y. and Moore, L. A. Studies on radiation-induced leukemia in mice. *Cancer Res.*, 19: 316, 1959.
37. Gross, L. Attempt to recover filterable agent from x-ray-induced leukemia. *Acta haemat.*, 19: 353, 1958.
38. Gross, L. Serial cell-free passage of a radiation-activated mouse leukemia agent. *Proc. Soc. Exper. Biol. & Med.*, 100: 102, 1959.
39. Lieberman, M. and Kaplan, H. S. Leukemogenic activity of filtrates from radiation-induced lymphoid tumors of mice. *Science*, 130: 387, 1959.
40. Holland, J. J., McLaren, L. C., Hoyer, B. H. and Syverton, J. T. Studies on infectious RNA from purified poliovirus and Coxsackie virus. *Fed. Proc.*, 19: 386, 1960; *ibid.* The mammalian cell virus relationship. v. Studies on the nature of enterovirus infectious ribonucleic acid. In preparation.
41. Miller, J. F. A. P. Fate of subcutaneous thymus grafts in thymectomized mice inoculated with leukaemic filtrate. *Nature, London*, 184: 1809, 1959.
42. Friend, C. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exper. Med.*, 105: 307, 1957.
43. Graffi, A. Chloroleukemia of mice. *Ann. New York Acad. Sc.*, 68: 540, 1957.
44. Friend, C. A virus-induced leukemia of adult mice. In: Perspectives in Virology, chap. 12B, p. 231. Edited by Pollard, M. New York, 1959. John Wiley & Sons, Inc.
45. Metcalf, D., Furth, J. and Buffett, R. F. Pathogenesis of mouse leukemia caused by Friend virus. *Cancer Res.*, 19: 52, 1959.
46. Sinkovics, J. G., Spurrier, W. A. and Schwartz, S. O. Studies in leukemia. xii. Interference and reactivation experiments with cell-free tissue extracts of leukemia Swiss mice. *Blood*, 15: 95, 1960.
47. Stewart, S. E. and Eddy, B. E. Properties of a tumor-inducing virus recovered from mouse neoplasms. In: Perspectives in Virology, chap. 12C, p. 245. Edited by Pollard, M. New York, 1959. John Wiley & Sons, Inc.
48. Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G. and Grubbs, G. E. The induction of neoplasms with a substance released from mouse tumors by tissue culture. *Virology*, 3: 380, 1957.
49. Sachs, L., Fogel, M. and Winocour, E. *In vitro* analysis of a mammalian tumour virus. *Nature, London*, 183: 663, 1959.
50. Rabson, A. S. and Legallais, F. Y. Cytopathogenic effect produced by polyoma virus in cultures of milk-adapted murine lymphoma cells (strain P388 D_1). *Proc. Soc. Exper. Biol. & Med.*, 100: 229, 1959.
51. Eddy, B. E., Stewart, S. E. and Berkeley, W. Cytopathogenicity in tissue cultures by a tumor virus from mice. *Proc. Soc. Exper. Biol. & Med.*, 98: 848, 1958.
52. Winocour, E. and Sachs, L. A plaque assay for the polyoma virus. (Letter to the Editor.) *Virology*, 8: 397, 1959.
53. Dulbecco, R. and Freeman, G. Plaque production by the polyoma virus. (Letter to the Editor.) *Virology*, 8: 396, 1959.
54. Stewart, S. E., Eddy, B. E. and Borgese, N. Neoplasms in mice inoculated with a tumor agent carried in tissue culture. *J. Nat. Cancer Inst.*, 20: 1223, 1958.
55. Eddy, B. E., Stewart, S. E., Stanton, M. F. and Marcotte, J. M. Induction of tumors in rats by tissue culture preparations of SE polyoma virus. *J. Nat. Cancer Inst.*, 22: 161, 1959.
56. Eddy, B. E., Stewart, S. E., Young, R. and Mider, G. B. Neoplasms in hamsters induced by mouse tumor agent passed in tissue culture. *J. Nat. Cancer Inst.*, 20: 747, 1958.
57. Eddy, B. E., Stewart, S. E., Kirschstein, R. L. and Young, R. D. Induction of subcutaneous nodules in rabbits with the SE polyoma virus. *Nature, London*, 183: 766, 1959.
58. McCulloch, E. A., Howatson, A. F., Siminovitch, L., Axelrad, A. A. and Ham, A. W. A cytopathogenic agent from a mammary tumour in a C3H mouse that produces tumours in Swiss mice and hamsters. *Nature, London*, 183: 1535, 1959.
59. Eddy, B. E., Stewart, S. E. and Grubbs, G. E. Influence of tissue culture passage, storage, temperature and drying on viability of SE polyoma virus. *Proc. Soc. Exper. Biol. & Med.*, 99: 289, 1958.
60. Brodsky, I., Rowe, W. P., Hartley, J. W. and Lane, W. T. Studies of mouse polyoma virus infection. ii. Virus stability. *J. Exper. Med.*, 109: 439, 1959.
61. Rowe, W. P., Hartley, J. W., Brodsky, I. and Huebner, R. J. Complement fixation with a mouse tumor virus (S. E. polyoma). *Science*, 128: 1339, 1958.
62. Eddy, B. E., Rowe, W. P., Hartley, J. W., Stewart, S. E. and Huebner, R. J. Hemagglutination

with the SE polyoma virus. (Letter to the Editor.) *Virology*, 6: 290, 1958.

63. Hartley, J. W. and Rowe, W. P. "Unmasking" of mouse polyoma virus hemagglutinin by heat and RDE. (Letter to the Editor.) *Virology*, 7: 249, 1959.
64. Rowe, W. P., Hartley, J. W., Estes, J. D. and Huebner, R. J. Studies of mouse polyoma virus infection. I. Procedures for quantitation and detection of virus. *J. Exper. Med.*, 109: 379, 1959.
65. Hartley, J. W., Rowe, W. P., Chanock, R. M. and Andrews, B. E. Studies of mouse polyoma virus infection. IV. Evidence for mucoprotein erythrocyte receptors in polyoma virus hemagglutination. *J. Exper. Med.*, 110: 81, 1959.
66. Kahler, H., Rowe, W. P., Lloyd, B. J. and Hartley, J. W. Electron microscopy of mouse parotid tumor (polyoma) virus. *J. Nat. Cancer Inst.*, 22: 647, 1959.
67. Negroni, G., Dourmashkin, R. and Chesterman, F. C. A "polyoma" virus derived from a mouse leukaemia. *Brit. M. J.*, 32: 1359, 1959.
68. Stanton, M. F., Stewart, S. E., Eddy, B. E. and Blackwell, R. H. Oncogenic effect of tissue culture preparations of polyoma virus on fetal mice. *J. Nat. Cancer Inst.*, 23: 1441, 1959.
69. Mirand, E. A., Mount, D. T., Moore, G. E., Grace, J. T., Jr. and Sokal, J. E. Induction of tumors by a virus-like agent(s) released by tissue culture. *Proc. Soc. Exper. Biol. & Med.*, 99: 1, 1958.
70. Habel, K. and Atanasiu, P. Transplantation of polyoma virus induced tumor in the hamster. *Proc. Soc. Exper. Biol. & Med.*, 102: 99, 1959.
71. Sachs, L. and Winocour, E. Formation of different cell-virus relationships in tumour cells induced by polyoma. *Nature, London*, 184: 1702, 1959.
72. Stewart, S. E. and Eddy, B. E. Tumor induction by SE polyoma virus and the inhibition of tumors by specific neutralizing antibodies. *Am. J. Public Health*, 49: 1493, 1959.
73. Dawe, C. J. and Law, L. W. Morphologic changes in salivary-gland tissue of the newborn mouse exposed to parotid-tumor agent in vitro. *J. Nat. Cancer Inst.*, 23: 1157, 1959.
74. Love, R. and Rabson, A. S. Cytochemical studies of milk-adapted murine lymphoma cells (strain P388 D_1) infected with polyoma virus. *J. Nat. Cancer Inst.*, 23: 875, 1959.
75. Law, L. W., Dawe, C. J., Rowe, W. P. and Hartley, J. W. Antibody status of mice and response of their litters to parotid tumour virus (polyoma virus). *Nature, London*, 184: 1420, 1959.
76. Rowe, W. P., Hartley, J. W., Brodsky, I., Huebner, R. J. and Law, L. W. Observations on the spread of mouse polyoma virus infection. *Nature, London*, 182: 1617, 1958.
77. Rowe, W. P., Hartley, J. W., Law, L. W. and Huebner, R. J. Studies of mouse polyoma virus infection. III. Distribution of antibodies in laboratory mouse colonies. *J. Exper. Med.*, 109: 449, 1959.
78. Meadors, G. F. Epidemiology of leukemia—PHR review. *Pub. Health Rep.*, 71: 103, 1956.
79. Lewis, E. B. Leukemia and ionizing radiation. *Science*, 125: 965, 1957.
80. MacMahon, B. Geographic variation in leukemia mortality in the United States. *Pub. Health Rep.*, 72: 39, 1957.
81. Gilliam, A. G. and Walter, W. A. Trends in mortality from leukemia in the United States, 1921–1955. *Pub. Health Rep.*, 73: 773, 1958.

The Metabolism of the Leukemic Leukocyte*

WILLIAM N. VALENTINE, M.D.

Los Angeles, California

THE problems of investigating leukocyte metabolism are indeed complex. Initially there must be a painstaking accumulation of descriptive data to define, insofar as possible, the metabolic profile of the human leukocyte. The data must be segregated in terms of the various morphologic cell types, since *a priori* these can be expected to differ from one another in terms of their biochemical constitution as well as in their functions in the body economy. It is also apparent that the operation of intracellular metabolic machinery will not be identical in cells of the same series in various stages of maturity and differentiation. The human leukocyte does not live in splendid isolation, and is responsive to changes in its hormonal environment, the availability and distribution of metabolically important substrates and modifiers, and to a host of largely unknown variables capable of influencing its metabolism. Morphologically identical cells can and do exhibit striking biochemical variations in the presence of disease. They may also bear the stamp of genetically determined inborn errors in metabolism [*1–3*]. Further, metabolic sequences involving multienzyme systems are exceedingly complex, and their direction and the rate at which they proceed are influenced by many factors which cannot necessarily be present during *in vitro* analyses. Analyses are further complicated by structural disorientations when intact cells cannot be employed, and by possible structural damage occurring during the isolation and handling of leukocyte material. It is therefore imperative to interpret *in vitro* data as an expression of metabolic activity applicable only under the precise conditions of the experiment.

Nonetheless, these inherent problems, which apply to metabolic and enzymologic investigations in general, do not preclude obtaining valuable and fundamental information concerning the intracellular machinery and its alterations in disease. Leukocytes are nucleated, readily available cells which may be biopsied repeatedly with safety and facility. Present methods of separation and proper controls permit the analyses of cell populations whose numbers may be accurately determined, and whose membership is limited to relatively few cell types in proportions which can be readily ascertained. Comparisons under standard conditions can be made of different cell populations in disease, and different metabolic patterns detected when these exist. It is becoming increasingly recognized that both conventional morphology and study of metabolism at the cellular level provide supplementary parameters in the investigation of leukocyte pathophysiology.

The application of enzymologic and biochemical technics to the problem of leukemia rests squarely on the assumption that abnormalities in cell growth, differentiation and behavior should be mirrored in an altered cell metabolism. In this sense, irrespective of whether leukemia is initiated by a virus, by genetic alterations produced by radiant energy, drugs, or unknown factors, or by changes in extracellular environmental factors, metabolic aberrations should appear as a reflection of the underlying etiology. It can be hoped that with sufficient data and sophistication these aberrations may be attacked in terms of metabolically oriented therapy.

LEUKOCYTE ALKALINE PHOSPHATASE

Neutrophilic granulocytes contain a highly labile phosphomonoesterase which has been the subject of extensive investigation. This intracellular enzyme, whose activity bears no discernible relationship to serum alkaline phosphatase levels, may be demonstrated by histochemical [*4,5*] and quantitative technics [*6,7*]. The former permit a qualitative or semiquantitative estimation of activity in individual cells;

* From the Department of Medicine, University of California Medical Center at Los Angeles, and the Wadsworth Hospital, V. A. Center, Los Angeles, California. Certain of the studies reported were supported by grants from the U. S. Public Health Service, Parke, Davis & Company, by contributions from Leukemia Research Foundation, and the Donate-Once Club of North American Aviation Corp., Los Angeles, California.

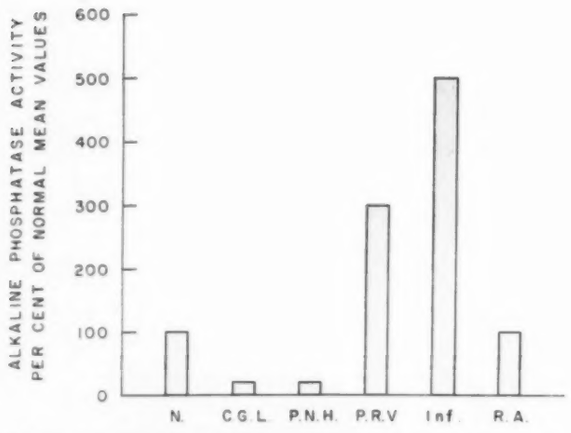


FIG. 1. A comparison of mean leukocyte alkaline phosphatase activity in groups of normal subjects and subjects with different disease states. The mean activity per 10^{10} leukocytes of normal subjects is set at 100 and variations in groups with disease are expressed as per cent of normal. N = Normal. C.G.L. = Chronic granulocytic leukemia. P.N.H. = Paroxysmal nocturnal hemoglobinuria. P.R.V. = Polycythemia rubra vera (selected cases with granulocytosis and/or leukemoid features). INF. = Miscellaneous pyogenic infections. R.A. = Rheumatoid arthritis.

the latter, a quantitative expression of activity in an isolated leukocyte preparation of known composition and numbers. When separated leukocyte populations from normal subjects are incubated at 37°C. and at pH 9.9 with sodium β-glycerophosphate as substrate, the mean activity may be expressed as approximately 25 mg. of phosphorus liberated from substrate per hour by 10^{10} leukocytes [*7*]. The normal range is approximately 10 to 50, similarly expressed. Marked species differences [*8,9*] exist, but in man the enzyme makes its appearance at about the myelocyte stage of development in the neutrophilic granulocytic series, increases with progressive maturation of the cell, and is largely or entirely absent from basophils, eosinophils and mononuclear leukocytes [*9*]. The enzyme is strikingly labile in the presence of disease. In chronic granulocytic leukemia both histochemical [*9,10*] and quantitative assays [*7,11*] ordinarily show very low activity. Histochemical methods indicate that this is true even in the morphologically mature cells [*9,12*]. At first glance it might be presumed that this low activity pattern reflects a generally lower level of cell maturity in the leukemic process. However, the marked increase in activity *above normal* demonstrable in comparably immature and morphologically indistinguishable populations from certain cases of classic polycythemia vera [*9,11,13*] and from some, although not all, metaplastic leukemoid reactions not regarded as leukemic [*9,11*] clearly indicates that this is not the case. It is therefore apparent that striking metabolic differences exist in cell populations of certain primary "myeloproliferative" disorders when differences are not demonstrable on morphological grounds alone. Figure 1 summarizes differences in mean alkaline phosphatase activity of leukocytes in various disease states.

These biochemical observations raise some interesting questions regarding certain as yet unsolved problems in hematology. There has long been controversey as to the relationship of chronic granulocytic leukemia, polycythemia vera with granulocytic leukemoid features, and a wide variety of primary metaplastic syndromes characterized by abnormal granulocytic blood pictures, varying erythrocyte and platelet aberrations, hepatosplenomegaly and extramedullary hematopoiesis. Some have favored the concept of a close etiologic relationship involving variable responses to a common stimulant; others favor the hypothesis that varying etiologies are operative, but that these give rise to superficially similar morphologic expressions. It may be pointed out in this latter regard that the primitive reticulum may theoretically respond to a number of stimuli by aberrant hematopoiesis involving erythrocytes, leukocytes and platelets in different degrees and by fibroblastic or osteosclerotic reactions. It is precisely these ingredients in varying combinations which are present in the myeloproliferative syndromes [*14*]. The issue is clouded by semantic problems regarding what constitutes "leukemia," which is identified on clinical, morphological and prognostic grounds. However, the ultimate problem, closely related to the development of rational therapy, resides in the identification of the etiology and pathophysiology of these clinical syndromes, and their separation on the basis of differing etiologies and pathophysiologic disturbances, if these exist.

The marked differences in leukocyte alkaline phosphatase in the myeloproliferative syndromes suggest but do not prove their heterogeneity. It has been said, for example, that many cases of polycythemia vera terminate as chronic granulocytic leukemia, the implication being that the two syndromes are merely variants from the start, or that, conversely, polycythemia may

change with time into a different entity. While transition is compatible with clinical observations, we have not yet observed metabolic transformation from a high to a low alkaline phosphatase pattern in polycythemia vera except in the relatively small proportion of cases in which a blastic picture develops. Blasts *per se* do not contain demonstrable alkaline phosphatase [*9*] activity in man. It is possible, therefore, that the transitional blood pictures represent only one end of the spectrum of polycythemia vera, a disease which in a majority of cases involves abnormalities of the granulocytes and platelets as well as the erythrocytes. It is only when these commonly encountered myelopoietic disturbances are present in severe or exaggerated form that transition to leukemia is suspected. It should be emphasized also that, while in many of the syndromes of myeloid metaplasia the high activity phosphatase pattern similar to that found in the leukemoid reactions of polycythemia vera is present, this is not true in all [*11,15–17*]. In certain cases, normal or low leukocyte alkaline phosphatase is observed. It may also be pointed out that in polycythemia vera without granuloctyosis or leukemoid features the phosphatase is usually in the normal range [*11*].

On the other hand, while such metabolic differences suggest heterogeneity, the point is not proved. It is not clear whether the differences in phosphatase are primary and fundamental or secondary and of minor significance. Values in the chronic granulocytic leukemic range may be found at times in other conditions, paroxysmal nocturnal hemoglobinuria being a prime example [*13,18*]. Alkaline phosphatase has been shown to be absent in the leukocytes of subjects with hypophosphatasia [*3*], an inborn error of metabolism, indicating that low values of the enzyme *per se* need not be associated with a leukemic syndrome. Further, while the alkaline phosphatase activity ordinarily remains low in therapeutically induced remissions in chronic granulocytic leukemia, exceptions may be noted in which it returns to normal [*19*]. This could be on the basis of a temporarily altered metabolic pattern in the leukemic cell. On the other hand, it could mean that occasionally remissions are so good that the normal cell lineage temporarily reasserts itself, and that at this time the cells whose activity is measured are a normal rather than a leukemic population. The latter "two population" hypothesis predicates that leukemia does not involve an over-all metabolic transformation in hematopoietic tissue, but rather overgrowth of the normal cell potential from a leukemic focus. It is also possible theoretically that the low phosphatase activity of granulocytes in chronic granulocytic leukemia reflects a dilution of cytoplasmic enzyme by abnormally rapid cell divisions at, for example, the progranulocyte-granulocyte stage of development. To explain high activity patterns in these terms would require the additional hypothesis that in certain other hyperplastic granulocytic states cell division may occur predominantly at a more primitive stage of cell development, but that new cells entering the granulocytic pool in this manner then mature with less dilution of certain cytoplasmic components through later stage cell divisions. These considerations are, of course, purely speculative and merely emphasize the need for more information.

Unit granulocyte alkaline phosphatase increases up to manyfold in a wide variety of clinically stressful circumstances such as pyogenic infection, myocardial infarction, surgery and diabetic acidosis [*20,21*]. On the other hand, symptomatic illness does not always elicit this response. In many collagen diseases, such as active rheumatoid arthritis, the leukocytes frequently exhibit normal alkaline phosphatase activity [*20*]. Normal activity has also been noted in the granulocytes of a number of cases of infectious mononucleosis in the presence of fever and symptomatic illness [*12,18a*]. The administration of large doses of ACTH or 17-hydroxycorticosteroids will increase per cell phosphatase activity markedly in a seventy-two-hour period [*21*]. In subjects with chronic granulocytic leukemia little or minimal response occurs under the same conditions. It thus appears that this leukocytic enzyme is responsive to changes in the activity of the pituitary-adrenal axis in nonleukemic subjects, but not to any appreciable extent in subjects with chronic granulocytic leukemia. The ultimate significance of these observations remains to be elucidated. Representative data are shown in Figure 2.

GLYCOLYSIS AND RESPIRATION

Lactic acid production by leukocytes has been recognized since Slosse [*22*] observed this metabolite to increase in blood on standing, and Levene and Meyer [*23*] studied the conversion of glucose to lactic acid in leukocytic exudates obtained in dogs. Grafe [*24*] in 1911 first studied

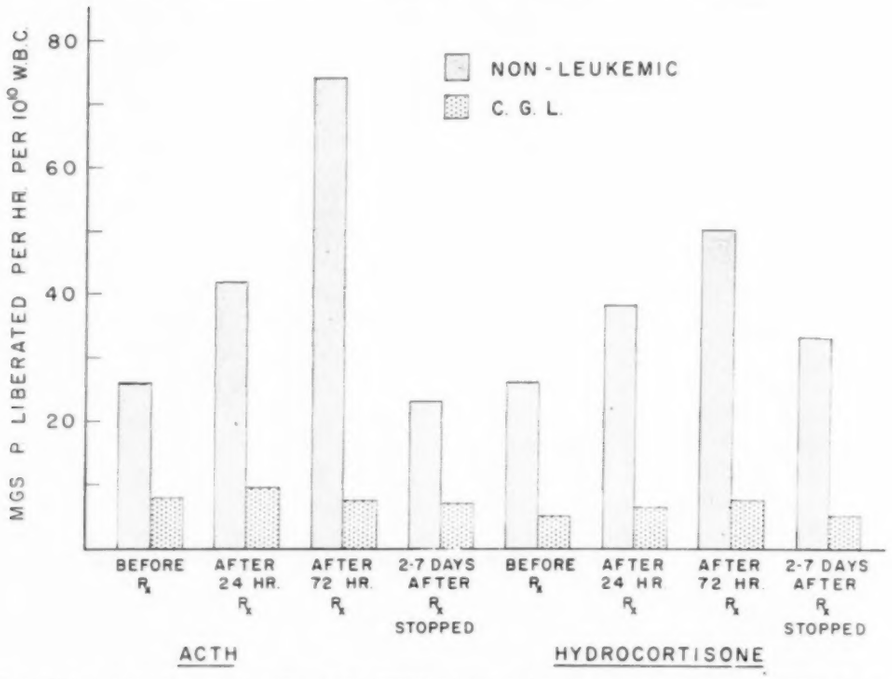


FIG. 2. A comparison of the effects of ACTH gel (40 units given intramuscularly every eight hours for nine doses) and hydrocortisone (60 to 70 mg. given orally every eight hours for nine doses) on leukocyte alkaline phosphatase in non-leukemic subjects and subjects with chronic granulocytic leukemia (C.G.L.). Mean values for seven non-leukemic and five leukemic subjects are graphed. Values are expressed as milligrams of phosphorus liberated per hour from the substrate sodium beta-glycerophosphate by 10^{10} leukocytes at 37°c.

the respiratory activity of leukocytes in an attempt to explain the marked increase in oxygen consumption in leukemic subjects. Numerous studies over the years have given controversial results difficult to evaluate comparatively because of widely differing source materials, varying *in vitro* systems and methods, and different reference standards for expressing data. The literature prior to 1953 has been reviewed by Beck and Valentine [*25*] in an effort to analyze materials and methods used, and to express certain of the available data in terms of the common denominator of cell numbers. The older and the more recent literature are in agreement that leukocytes from normal subjects and subjects with chronic granulocytic leukemia possess a substantial aerobic glycolysis, while those from subjects with chronic lymphocytic leukemia have a comparatively low glycolytic rate. In a homogenate system containing diphosphopyridine nucleotide (DPN) (0.0007M), adenosine triphosphate (ATP) (0.0011M), cytochrome c (1.4×10^{-5}M), phosphate (0.01M), Mg^{++} (0.005M) and substrates such as glucose and hexose diphosphate (HDP) (0.005M) at pH 7.4 and 37°c., striking differences between normal and leukemic leukocytes are noted in oxygen consumption, glucose utilization and lactic acid production [*26*]. Beck's recent data [*27*] indicate lactic acid production of 45.2, 13.2 and 4.8 μM. per 10^8 leukocytes per hour in normal, chronic granulocytic leukemic and chronic lymphocytic leukemic leukocytes, respectively. The incubation system contained glucose (0.005M). Oxygen consumption was observed to be 4, 1.2, and 1.1 μM. per 10^8 cells per hour and glucose utilization 21, 5.8, and 2.9 μM. per hour in the same three types of cell populations. (Fig. 3.) The findings indicate a predominantly aerobic glycolytic metabolism, the ratio of lactic acid production/glucose utilization, calculated in terms of glucose equivalents, being 36.8 in normal cell populations, 33 in chronic granulocytic leukemic cells, and 13.1 in chronic lymphocytic leukemic cells. With substrate and co-factor manipulation further differences were observed [*28*]. Succinate stimulated oxygen uptake to a greater extent in normal than in leukemic cell populations, while leukemic leukocytes required more cytochrome c

for maximal O_2 consumption than did normal cells. Homogenates of chronic lymphocytic leukemic cells required more DPN for maximal lactic acid production than did normal leukocytes.

The mechanism behind the significantly lower lactic acid production in leukemic leukocytes has been extensively studied by Beck [*27–30*] who has attempted to define the rate limiting reactions in normal and leukemic material by analyses of individual glycolytic enzymes. Several enzymes of the Embden-Meyerhof cycle were found to possess substantially lower activity in leukemic leukocytes. Hexokinase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase and lactic dehydrogenase activities were well below normal levels in chronic granulocytic leukemic cells when compared on the basis of activity per 10^{10} leukocytes. On the other hand, phosphohexose isomerase, aldolase and triosephosphate isomerase were entirely normal or slightly elevated in the chronic granulocytic leukemic leukocytes. (Table 1.) Although several enzymes were low in activity, a comparison of maximal capacity of individual enzymes and that of the over-all glycolytic system pointed to hexokinase as the rate limiting reaction in both normal and leukemic material, with phosphofructokinase a close second. The chronic granulocytic leukemic leukocytes, however, were comparatively hexokinase deficient, and additions of purified hexokinase increased over-all glycolysis 50 per cent in normal material and 300 per cent in leukemic material. The leukemic cells were also shown to be deficient in adenosine diphosphate (ADP) generating mechanisms, including not only those dependent on hexokinase and phosphofructokinase but also possibly others, such as adenosine triphosphatase (ATPase). When ADP was added to systems employing glucose as glycolyzable substrate, leukemic homogenates exhibited a greater percentage increase in glycolysis than did the normal system. Supplementation with ATPase resulted in a rise in lactic acid production in the chronic granulocytic leukemic system which was further potentiated by hexokinase additions. With normal cell homogenates only a slight rise in lactic acid production was observed with ATPase supplementation. Glucose metabolism in leukocytes chiefly resulted in formation of lactic acid via pyruvate, and lactic acid was demonstrated to be reutilized by radioisotopic technics. No difference in properties of normal and leukemic enzymes was demonstrable, the Michaelis constants and pH activity curves being analogous in each instance.

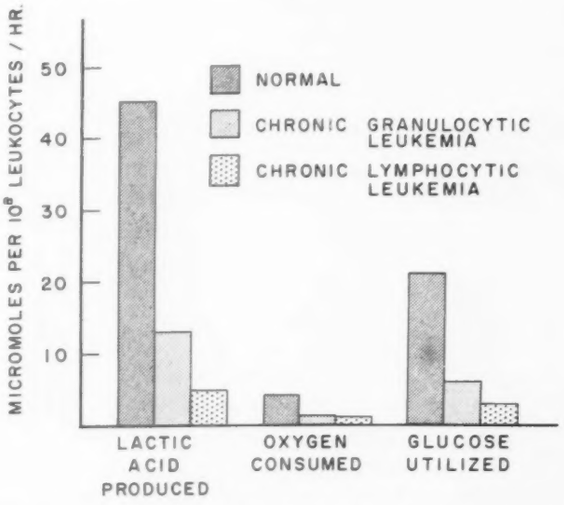


FIG. 3. A comparison of lactic acid production, oxygen consumption and glucose utilization in normal and leukemic homogenates. The incubation system is discussed in text. Values are means of more than sixty determinations in each case derived from the data of BECK, W. S. *Ann. N. Y. Acad. Sci.*, 75: 4, 1958 [*27*].

In investigations on the phosphogluconate pathway of glucose metabolism, Beck [*31*] has presented evidence indicating that glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity are reduced in cells of chronic leukemia. Phosphopentose isomerase activity was comparable in chronic granulocytic leukemic and normal cells. The dehydrogenases, however, were present in ten times greater concentration than necessary for their observed metabolic load. In the system employed, less than 10 per cent of glucose was metabolized via the phosphogluconate pathway, but the per cent was higher in leukemic cells. The levels of phosphogluconate pathway metabolism were nearly the same in intact cells as in homogenate systems. It is thus demonstrated that the metabolic pattern in leukemic cells is definitely abnormal and these aberrations may play a role, as yet not precisely defined, in the cell growth and differentiation abnormalities characteristic of the leukemic state.

Wagner [*32–34*] has made careful and valuable observations on the metabolism of glycogen and glucose in leukocytes, identifying the phosphorylated intermediates, and demonstrating that the breakdown of glycogen to lactic acid is the result of phosphorylative glycogenolysis. More

TABLE 1

COMPARISON OF MEAN OVER-ALL GLYCOLYTIC RATE AND MEAN RATE OF INDIVIDUAL ENZYME ACTIVITIES IN NORMAL, CHRONIC GRANULOCYTIC LEUKEMIC (C.G.L.) AND CHRONIC LYMPHOCYTIC LEUKEMIC (C.L.L.) HOMOGENATES*

Activity	Per Cent of Normal	
	C.G.L.	C.L.L.
1. Overall aerobic glycolysis	29	11
2. Glucose $\xrightarrow{\text{hexokinase}}$ glucose-6-phosphate	47	17
3. Glucose-6-phosphate $\xrightarrow[\text{phosphohexose isomerase}]{}$ fructose-6-phosphate	100	26
4. Fructose-6-phosphate $\xrightarrow[\text{phosphofructokinase}]{}$ fructose-1,6-diphosphate	42	17
5. Fructose-1,6-diphosphate $\xrightarrow[\text{aldolase}]{}$ dihydroxy acetone phosphate + D-glyceraldehyde-3-phosphate	107	35
6. Dihydroxyacetone phosphate $\xrightarrow[\text{triosephosphate isomerase}]{}$ D-glyceraldehyde-3-phosphate	106	32
7. Phosphoenolpyruvic acid $\xrightarrow[\text{pyruvate kinase}]{}$ pyruvic acid	37	13
8. Pyruvic acid $\xrightarrow[\text{lactic dehydrogenase}]{}$ lactic acid	41	24

* From data of BECK, W. S. *J. Biol. Chem.*, 232: 251, 1958.

The data indicate marked percentile reductions in activity of a number of enzymes in chronic leukemic cells when compared to normal. The mean normal value in each instance is set as 100, and the values in chronic leukemia expressed as per cent of normal.

recently this investigator and his associates have published observations on endogenous respiration of isolated leukocytes made on dialyzed and undialyzed cells [*35,36*]. In the absence of added substrates endogenous leukocyte respiration was reduced almost to zero by dialysis, whereas without dialysis significant oxygen uptake occurred. In horse leukocytes, α-glycerophosphate rather than succinate appeared to be the principal substrate for endogenous oxygen uptake. After dialysis of leukocyte suspensions, oxygen uptake could be markedly increased by addition of α-glycerophosphate to the suspending medium, but the increase in chronic granulocytic leukemic material was but 25 per cent of that in normal cell populations. In normal cells, additions of cytochrome c potentiated respiration in the presence of α-glycerophosphate in both dialyzed and undialyzed suspensions. However, in chronic granulocytic leukemic leukocytes there was no increase in α-glycerophosphate dehydrogenase activity following addition of cytochrome c to the reaction mixture. Wagner points out that it cannot yet be assumed that α-glycerophosphate dehydrogenase activity is low in these leukemic leukocytes, since this behavior could result from defects in any of a series of enzymes necessary to obtain oxygen uptake. Quantitative studies on cytochrome were contemplated.

OTHER ENZYMES

Myeloperoxidase. Granulocytic leukocytes are rich in a green hemoprotein, peroxidative enzyme known as myeloperoxidase or verdoperoxidase [*37–39*]. This green enzyme is particularly rich in chloroma tissue, giving it the characteristic green color [*37*]. Myeloperoxidase can oxidize reduced diphosphopyridine nu-

cleotide (DPNH) rapidly in the presence of hydrogen peroxide, but the precise role of this peroxidative catalyst in the leukocyte is unknown [*40*].

Beta Glucuronidase. This enzyme, which is capable of hydrolysing and perhaps in certain circumstances of synthesizing glucuronide conjugates, is present in considerable amounts in human leukocytes [*41,42*]. It is generally agreed that the lymphocytes of chronic lymphocytic leukemia are low in this activity [*43,44*], and Follette et al. [*44*] have observed low values in non-leukemic lymphocytoses as well. Controversial results have been obtained in acute leukemia and in chronic granulocytic leukemia. Anlyan and his associates [*43*] found low values in chronic granulocytic leukemic leukocytes and normal activity in blast cell populations. In the author's laboratory [*44*] chronic granulocytic leukemic cells were found to contain normal or somewhat elevated activity, whereas low values were noted in blastic exacerbations of chronic granulocytic leukemia or in acute blastic leukemia *per se*. The reason for these differences is not entirely clear, nor is the metabolic function of this enzyme in leukocytes apparent, particularly since hydrolytic rather than synthetic activity appears the more likely primary function of the enzyme.

Esterase and Lipase. Enzymes capable of hydrolysing esters of both short chain and long chain fatty acids are found in human leukocytes [*45,46*], and appear to be distributed ubiquitously throughout members of both the granulocytic and mononuclear cell series [*46*]. No characteristic variations in leukemia have been demonstrated, however.

Glyoxalase. Although no important metabolic function has been assigned to this enzyme, and although its substrate, methyl glyoxal, has not been satisfactorily demonstrated as a metabolic intermediate of any significance, both leukocytes and erythrocytes contain it. McKinney [*47*] has reported an activity several hundredfold greater in the leukocyte than the erythrocyte on a per cell basis, and has postulated that glyoxalase could be involved in an alternative pathway to lactic acid. Observations in our laboratory [*48*] confirm the presence of glyoxalase in leukocytes but fail to confirm the high leukocyte/erythrocyte glyoxalase ratio reported by McKinney. McKinney [*47*] has reported diminished glyoxalase in the leukocytes of chronic granulocytic leukemia.

Acid Phosphatase [*6,7,13*]. Blast cells and lymphocytes of chronic lymphocytic leukemia are low in acid phosphastae [*13*], whereas values slightly above the normal mean are found in chronic granulocytic leukemia [*7*] and in certain cases of polycythemia rubra vera with leukemoid features.

Arginase and Arginosuccinase. These enzymes of the Krebs-Hensleit urea cycle are present in human leukocytes [*18b,49*]. No characteristic patterns have been demonstrated in the leukemias.

Enzymes in Pyrimidine Synthesis. Employing isotopic technics, disrupted cells, and micro assay procedures, three enzymes leading to formation of orotic acid, a known important intermediate in the formation of nucleic acid, have been demonstrated in leukocytes by Smith and Baker [*50*]. Aspartate carbamyltransferase, dehydro-orotase and dehydro-orotic dehydrogenase activity were demonstrated in circulating leukocytes, and the intracellular locations and biochemical characteristics of these enzymes defined. Comparative studies with leukemic material are not yet available.

Arylsulfatase. More than a thirtyfold increase in this enzyme has been found in the urine of patients with chronic granulocytic leukemia [*51*]. The enzyme activity is low in lymphocytes of chronic lymphocytic leukemia [*18b*]. Activity in the leukocytes of chronic granulocytic leukemia is normal or elevated, high activity being usually associated with increases in the eosinophils and basophils. The highest activities thus far observed have been in cell populations characterized by marked eosinophilia, although elevated values were also found in a leukemic subject with 60 to 70 per cent basophils [*18b*].

Others. Needless to say, the preceding summarization does not include all the manifold enzymologic studies touching on leukocyte metabolism. Proteolytic ferments, various dehydrogenases, transaminase, catalase and many other enzymes are known to be present. Activities in the various leukemias, however, are not adequately documented as yet.

NON-ENZYMATIC CONSTITUENTS

Histamine. The early work of Code demonstrated the presence of a substance pharmalogically identical with histamine in human leukocytes [*52*]. Marked elevations have been observed in the leukocytes of chronic granulocytic leukemia [*53–55*], and evidence now points to the basophil, frequently found in increased

TABLE II
COMPARISON OF HISTAMINE IN MICROGRAMS OF FREE BASE PER 10^8 GRANULOCYTES IN TWENTY-TWO DETERMINATIONS IN SUBJECTS WITH CHRONIC GRANULOCYTIC LEUKEMIA

Group	Determinations (no.)	Per Cent Basophils		Histamine*	
		Mean	Range	Mean	Range
B > 8%	8	29.4	9.6–67.1	21.8	5.1–76.7
B < 8%	14	4.1	1.7– 7.1	3.8	1.9– 6.4

* Histamine expressed as micrograms of free base per 10^8 granulocytes.

numbers in this disease, as the principal repository of histamine in man [*56–58*]. The basophil is thus similar in this respect to the tissue mast cell, with its similar metachromatically staining granulations [*59,60*]. Histamine may be present in lesser degree in other leukocytes such as the eosinophil [*57*]. We have observed in a leukemic subject, with large numbers of circulating basophils, pharmacologically bound leukocytic histamine equivalent to the astonishingly large amount of 200 mg. in the total circulating blood volume. (Table II.)

Sulfhydryl Compounds. Employing the nitroprusside method for glutathione determination, normal leukocytes have been shown to contain 3.3 to 6.3 mg. per 10^{10} cells [*61*]. Values in certain blastic leukemias and in chronic lymphocytic leukemia were lower on a per cell basis. Employing an amperometric method measuring other non-protein sulfhydryl constituents as well as glutathione, Green and Martin [*62*] observed increases in soluble sulhydryl content in leukemic leukocytes. Contopoulos and Anderson [*63*] used a different method, the iodometric technic, and found increased values in a limited number of subjects with chronic lymphocytic, chronic granulocytic and acute leukemia. The differing results probably reflect differences in methodology. Ergothioneine, a sulfhydryl constituent of blood cells, and ascorbic acid do not interfere in the nitroprusside method. Weisberger and his associates [*64–66*] have extensively studied cysteine and sulfur metabolism in normal and leukemic material. Radioactive L-cysteine is more rapidly incorporated into acute and chronic granulocytic leukemic cells than into normal leukocytes. L-cysteine appears to have a specific protective modifying effect on nitrogen mustard-induced leukopenia [*66*], and this effect is dependent on spatial orientations as well as the presence of sulfhydryl, amino and carboxyl groups in close apposition.

Glycogen. The distribution of glycogen among the various formed elements of the blood is still open to some controversy. Wagner's work [*67,68*] on the quantitative estimation of glycogen in human blood indicates that glycogen is essentially limited to the granulocytic elements, and this worker concludes that reducing substances found in lymphocytes are not glycogen. High values were noted in the granulocytes of polycythemia, while in chronic granulocytic leukemia the values were in the normal range for the most part. Studies in this laboratory [*11,69*], employing the anthrone method of estimation after alkali digestion and alcohol precipitation, confirmed the high values in polycythemia, but also pointed to lower mean levels of glycogen per 10^{10} granulocytes in chronic granulocytic leukemia. The mean glycogen content per 10^{10} myeloid leukocytes was 75.1 mg. in normal subjects, 37.9 mg. in subjects with chronic granulocytic leukemia, and 116.2 mg. in fifteen subjects with polycythemia and marked leukemoid blood pictures. Values in acute blastic and chronic lymphocytic leukemia were very low; in one subject with chronic lymphocytic leukemia and essentially 100 per cent lymphocytes no measurable glycogen could be recovered. Wislocki and associates [*70*], employing the periodic acid-Schiff reaction histochemically and saliva digestion to differentiate glycogen from other reacting substances, found only one of ten lymphocytes to contain positive granules, and these did not seem to be soluble in saliva. Gibb and Stowell [*71*], using three different histochemical technics and the saliva digestion test, found large amounts of glycogen in neutrophilic and eosinophilic granulocytes and smaller amounts in lymphocytes. They estimate that a large number of blasts or lymphocytes, "possibly a hundred or more," would be required to contain glycogen equivalent to one polymorphonuclear leukocyte. Wachstein [*72*] found only occasional histochemically demonstrable glycogen in normal or lymphocytic leukemic lymphocytes, absent glycogen in films from normal lymph nodes, and increasingly large amounts of glycogen in myeloid granulocytes from the myelocyte stage of development to more mature cells. On the other hand, Astaldi and Verga [*73*], and more recently

Mitus and associates [*74*], have reported an increase in histochemically demonstrable glycogen in chronic lymphocytic leukemia and lymphosarcoma. A malt diastase preparation was employed to distinguish glycogen in the latter study. The reasons for certain discrepancies in results are not entirely clear. One possible source of difference lies in the problems of quantitative and histochemical methodology. Another lies in the fact that glycogen exists in varying degrees of polymerization [*75*], and conceivably varying solubility characteristics of different glycogens may influence quantitative recovery data.

Glucuronic Acid. Leukocytes contain glucuronic acid [*76*], the mean value being 3.7 mg. per 10^{10} leukocytes in mixed cell, normal populations. Essentially comparable although minimally lower mean values are observed in chronic granulocytic leukemia, while blast cells and chronic lymphocytic leukemic leukocytes have a low per cell content of this constituent. The lymphocyte, it must be remembered, is a small cell, and the values for this and other lymphocytic constituents are appreciably higher on a cell nitrogen than on a cell number reference basis.

Heparin. It has been claimed [*77*] that heparin is extractable from the blood basophil, suggesting another close analogy of this blood constituent and the tissue mast cell [*60*].

Zinc. Vallee [*78*] has recently reviewed the literature on the biochemistry, physiology and pathology of zinc. Leukocytes are said to contain about twenty-five times as much zinc per cell as do erythrocytes [*79*], and a protein containing 0.3 per cent zinc per gram of dry weight can be obtained from human leukocytes [*80,81*]. The possible enzymatic nature of this material is unknown. Wolff [*82*] reported leukocyte zinc values of 2.3×10^{-2} μg. per 10^6 cells. The zinc concentration per leukocyte is reported to be decreased to as little as 10 per cent of the normal value in certain cases of chronic granulocytic leukemia in relapse. With urethane or x-ray therapy and remission, the values returned to normal. The intravenous administration of zinc did not influence leukocyte zinc concentration or the course of the disease [*83*]. There is evidence that porcine kidney phosphatase contains zinc [*84*]. Zinc, 10^{-4}M, is much more effective than Mg^{++} in restoring activity to leukocyte alkaline phosphatase inactivated by EDTA [*85*]. Storage of leukocytes in saline solution containing Zn^{++} 10^{-4}M largely prevents or mitigates loss of activity, and Zn^{++} is much more effective than Mg^{++} in partially restoring activity lost on storage in saline solution in the absence of the metal [*86*]. There is, then, a suggestion that Zn^{++} may play a significant role in this labile, intracellular leukocytic enzyme.

Amino Acids. Total amino acid nitrogen has been reported [*87*] to be lower in chronic lymphocytic and granulocytic leukemia, monocytic leukemia and acute leukemia than in normal subjects. Serine, glycine, taurine, β-aminoethyl phosphate, alanine, threonine, leucine(s), glutamic and aspartic acids have been identified in leukocytes as free intracellular amino compounds. Glutamic acid was highest in cells of acute leukemia and lowest in normal cells. The reverse was true for taurine. β-aminoethyl phosphate was highest in chronic lymphocytic leukemic lymphocytes. These three compounds together with serine and glycine accounted for two-thirds of the total free amino nitrogen. Iyer [*88*] has reported that leukocytes in chronic granulocytic leukemia contain greater than normal amounts of alanine, aspartic and glutamic acids, glutamine, glycine, valine, leucine, isoleucine and taurine. Taurine is present in high concentration in leukocytes. It is not known whether the differences in leukemia are related to rapid proliferation *per se* or have a more specific significance. Also, inadequate data are available in terms of the different morphological composition of cell populations analysed. Further, Rouser has emphasized that interrelationships exist between plasma and cellular levels of free amino acids, and that a careful comparison of amino acid distribution in cells, plasma and urine is necessary in order to evaluate properly any changes observed [*89*].

SUMMARY AND CONCLUSIONS

It is apparent that the metabolism and the enzymatic and biochemical constitution of human leukocytes vary in different normal morphological cell types, in normal leukocytes of the same series but in various stages of differentiation, and in morphologically identical leukocytes present in leukemia, in non-leukemic disorders and in normal subjects. Quantitative differences are demonstrable between individual enzymes and non-enzymatic constituents of normal and leukemic cells, and also in metabolic

activities dependent upon sequential reactions in multienzyme systems. In the case of leukocyte alkaline phosphatase, the usual pattern is one of low activity, while in comparably immature and essentially morphologically similar cell populations in polycythemia vera, high activity patterns are the rule. In myeloid metaplasia the more common pattern of activity resembles that seen in polycythemia vera, but low activities resembling that of chronic granulocytic leukemia may also be observed at times. This suggests but does not prove the heterogeneity of myeloid metaplasia in terms of pathophysiology. Exceptions to the usually observed patterns may occur and these are discussed. Normal leukocytes respond to many naturally occurring "stresses" and to large doses of exogenously administered ACTH or 17-OH-corticosteroids by substantial elevations in unit cell alkaline phosphatase activity. This response is evoked minimally, if at all, in most subjects with chronic granulocytic leukemia.

In terms of glycolysis and respiration, chronic leukemic leukocytes utilize less glucose, consume less oxygen, and produce less lactic acid than their normal counterparts in fortified homogenate systems. In both normal and chronic granulocytic leukemic cells such homogenate systems demonstrate a predominantly aerobic glycolytic metabolism, the ratio of lactic acid produced to oxygen consumed being high. There are quantitative differences between normal and chronic leukemic cells in the maximum capacities of individual enzymes of the glycolytic cycle. However, hexokinase appears the most likely rate limiting glycolytic enzyme step in both normal and chronic leukemic material. The enzyme is, however, relatively deficient in the latter. Evidence also exists that adenosine diphosphate (ADP) generating mechanisms are deficient in general in chronic leukemic cells. Numerous other enzymatic and non-enzymatic constituents have also been investigated in normal and leukemic leukocytes. In many instances the data are somewhat fragmentary and descriptive, permitting little in the way of generalization. In some, the results are controversial and future studies are required to resolve differences in the observations thus far available. Some of these constituents are discussed in descriptive terms in this report. While the problems are complex and the possible pitfalls in methodology and interpretation numerous, it is to be hoped that a better understanding of leukocyte metabolism at the level of the cell will give rise to a better understanding of the aberrations in cell growth, differentiation and behavior characteristic of the leukemic state.

REFERENCES

1. Wagner, R. Glycogen content of isolated white blood cells in glycogen storage disease. *Am. J. Dis. Child.*, 73: 559, 1947.
2. Bridge, E. N. and Holt, L. E. Glycogen storage disease: observations on the pathologic physiology of two cases of the hepatic form of the disease. *J. Pediat.*, 27: 299, 1945.
3. Kretchmer, N., Stone, M. and Bauer, C. Hereditary enzymatic effects as illustrated by hypophosphatasia. *Ann. New York Acad. Sc.*, 75: 279, 1958.
4. Gomori, G. Microchemical demonstration of phosphatase in tissue sections. *Proc. Soc. Exper. Biol. & Med.*, 42: 23, 1939.
5. Kaplow, L. S. A histochemical procedure for localizing and evaluating leukocyte alkaline phosphatase activity in smears of blood and marrow. *Blood*, 10: 1023, 1955.
6. Haight, W. F. and Rossiter, R. J. Acid and alkaline phosphatase in white cells. Data for the lymphocyte and the polymorphonuclear leukocyte of man and the rabbit. *Blood*, 5: 267, 1950.
7. Valentine, W. N. and Beck, W. S. Biochemical studies on leukocytes. I. Phosphatase activity in health, leucocytosis and myelocytic leucemia. *J. Lab. & Clin. Med.*, 38: 39, 1951.
8. Moloney, W. C. Leukocyte alkaline phosphatase activity in the rat. *Ann. New York Acad. Sc.*, 75: 31, 1958.
9. Wachstein, M. Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. *J. Lab. & Clin. Med.*, 31: 1, 1946.
10. Plum, C. M. Die alkalische Phosphatase in den Zellen des normalen und pathologischen Knochenmarkes und des peripheren Blutes. *Acta haemat.*, 4: 73, 1950.
11. Valentine, W. N., Beck, W. S., Follette, J. H., Mills, H. and Lawrence, J. S. Biochemical studies in chronic myelocytic leukemia, polythemia vera and other idiopathic myeloproliferative disorders. *Blood*, 7: 959, 1952.
12. Kenny, J. J. and Moloney, W. C. Leukocyte alkaline phosphatase. Behavior during prolonged incubation and infection in normal and leukemic leukocytes. *Blood*, 12: 295, 1957.
13. Beck, W. S. and Valentine, W. N. Biochemical studies on leucocytes. II. Phosphatase activity in chronic lymphocytic leucemia, acute leucemia, and miscellaneous hematologic conditions. *J. Lab. & Clin. Med.*, 38: 245, 1951.
14. Dameshek, W. Editorial. Some speculations on the myeloproliferative syndromes. *Blood*, 6: 372, 1951.
15. Leonard, B. J., Israels, M. C. G. and Wilkinson, J. F. Myelosclerosis. A clinicopathological study. *Quart. J. Med.*, 26: 131, 1957.
16. Koler, R. D., Seaman, A. J., Osgood, E. E. and

VANBELLINGHEN, P. Myeloproliferative diseases. Diagnostic value of the leukocyte alkaline phosphatase test. *Am. J. Clin. Path.*, 30: 295, 1958.

17. MITUS, W. J., GHERARDI, G. J., MEDNICOFF, J. B. and DAMESHEK, W. Cytochemistry of myelofibrosis with myeloid metaplasia in relation to spleen changes. *Arch. Path.*, 67: 188, 1959.
18. (*a*) TANAKA, K. R., VALENTINE, W. N. and FREDRICKS, R. E. Disease states or clinical conditions associated with low leukocyte alkaline phosphatase. *New England J. Med.*, in press.
(*b*) TANAKA, K. R., VALENTINE, W. N. and FREDRICKS, R. E. Unpublished data.
(*c*) TANAKA, K. R., VALENTINE, W. N. and FREDRICKS, R. E. Studies on leukocytic and erythrocytic enzymes in paroxysmal nocturnal hemoglobinuria. *Clin. Research*, 8: 132, 1960.
19. VALENTINE, W. N., FOLLETTE, J. H., SOLOMON, D. H. and REYNOLDS, J. Biochemical and enzymatic characteristics of normal and leukemic leukocytes (with particular reference to leukocyte alkaline phosphatase). In: The Leukemias. Etiology, Pathophysiology, and Treatment, p. 457. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W., New York, 1957. Academic Press, Inc.
20. VALENTINE, W. N., FOLLETTE, J. H., HARDIN, E. B., BECK, W. S. and LAWRENCE, J. S. Studies on leukocyte alkaline phosphatase activity: relation to "stress" and pituitary-adrenal activity. *J. Lab. & Clin. Med.*, 44: 219, 1954.
21. VALENTINE, W. N., FOLLETTE, J. H., SOLOMON, D. H. and REYNOLDS, J. The relationship of leukocyte alkaline phosphatase to "stress," to ACTH, and to adrenal 17-OH-corticosteroids. *J. Lab. & Clin. Med.*, 49: 723, 1957.
22. SLOSSE, A. Étude sur la glycolyse aseptique dans le sang. *Arch. internat. physiol.*, 11: 153, 1911.
23. LEVENE, P. and MEYER, G. On action of leukocytes on glucose. *J. Biol. Chem.*, 12: 265, 1912.
24. GRAFE, E. Die Steigerung des Stoffwechsels bei chronischer Leukämie und ihre Ursachen (Zugleich ein Beitrag zur Biologie der weissen Blutzellen). *Deutsch. Arch. f. klin. Med.*, 102: 406, 1911.
25. BECK, W. S. and VALENTINE, W. N. The carbohydrate metabolism of leukocytes: a review. *Cancer Res.*, 13: 309, 1953.
26. BECK, W. S. and VALENTINE, W. N. The aerobic carbohydrate metabolism of leukocytes in health and leukemia. I. Glycolysis and respiration. *Cancer Res.*, 12: 818, 1952.
27. BECK, W. S. Leukocyte glycolysis; an investigation of the factors controlling the rate behaviour in multienzyme systems. *Ann. New York Acad. Sc.*, 75: 4, 1958.
28. BECK, W. S. and VALENTINE, W. N. The aerobic carbohydrate metabolism of leukocytes in health and leukemia. II. The effect of various substrates and coenzymes on glycolysis and respiration. *Cancer Res.*, 12: 823, 1952.
29. BECK, W. S. A kinetic analysis of the glycolytic rate and certain glycolytic enzymes in normal and leukemic leukocytes. *J. Biol. Chem.*, 216: 333, 1955.
30. BECK, W. S. The control of leukocyte glycolysis. *J. Biol. Chem.*, 232: 251, 1958.
31. BECK, W. S. Occurrence and control of the phosphogluconate oxidation pathway in normal and leukemic leukocytes. *J. Biol. Chem.*, 232: 271, 1958.
32. WAGNER, R. and REINSTEIN, S. Enzyme studies on white blood cells. II. Phosphorylating glycogenolysis. Accumulation of an intermediary reducing substance and formation of lactic acid. *Arch. Biochem.*, 29: 260, 1950.
33. WAGNER, R. and YOURKE, A. Enzyme studies on white blood cells. III. Phosphorylating glycogenolysis and phosphorylated intermediates. *Arch. Biochem.*, 39: 174, 1952.
34. WAGNER, R. and YOURKE, A. Enzyme studies on white blood cells. IV. Glycolysis of added glucose. Hexokinase and adenylpyrophosphatase activity. *Arch. Biochem.*, 44: 415, 1953.
35. WAGNER, R., MEYERRIECKS, N. and SPORACO, R. Enzyme studies on white blood cells and blood platelets. V. Dehydrogenase activity. *Arch. Biochem.*, 61: 278, 1956.
36. WAGNER, R. and SPORACO, R. Enzyme studies on white blood cells. *Ann. New York Acad. Sc.*, 75: 16, 1958.
37. AGNER, K. Verdoperoxidase. *Acta physiol. scandinav.* (Suppl. 8) 2: 1, 1941.
38. FOULKES, E. C., LEMBERG, R. and PURDOM, P. Verdohaem and verdoglobins. *Proc. Roy. Soc. London*, S.B 138: 386, 1951.
39. CHANCE, B. The cytochromes of respiring cells. In: Blood Cells and Plasma Proteins. p. 306. Edited by J. L. Tullis. New York, 1953. Academic Press, Inc.
40. SCHULTZ, J. Myeloperoxidase. *Ann. New York Acad. Sc.*, 75: 22, 1958.
41. ANLYAN, A. J. and FISHMAN, W. H. β-glucuronidase activity in human neoplastic tissues and in certain body fluids and secretions. *Bull. Am. Coll. Surgeons*, 32: 262, 1947.
42. FISHMAN, W. H., SPRINGER, B. and BRUNETTI, R. Application of an improved β-glucuronidase assay method for the study of human blood β-glucuronidase. *J. Biol. Chem.*, 173: 449, 1948.
43. ANLYAN, A. J., GAMBLE, J. and HOSTER, H. A. β-glucuronidase activity in human leukemias and Hodgkin's disease. *Cancer*, 3: 115, 1950.
44. FOLLETTE, J. H., VALENTINE, W. N. and LAWRENCE, J. S. The beta-glucuronidase content of human leukocytes in health and disease. *J. Lab. & Clin. Med.*, 40: 825, 1952.
45. AMMON, R. and JAARMA, J. Enzymes hydrolysing fats and esters. In: The Enzymes. Chemistry and Mechanism of Action. vol. 1, p. 390. Edited by Sumner, J. B. and Myrbäck, K. New York, 1950, Academic Press, Inc.
46. HARDIN, E. B., VALENTINE, W. N., FOLLETTE, J. H. and LAWRENCE, J. S. Esterase and lipase activity of leukocytes and erythrocytes in health and disease. *Am. J. M. Sc.*, 229: 395, 1955.
47. MCKINNEY, G. R. Glyoxalase activity in human leukocytes. *Arch. Biochem.*, 46: 246, 1953.
48. VALENTINE, W. N., TANAKA, K. R. and FREDRICKS, R. E. Unpublished data.
49. REYNOLDS, J., FOLLETTE, J. H. and VALENTINE, W. N. The arginase activity of erythrocytes and leukocytes with particular reference to pernicious anemia and thalassemia major. *J. Lab. & Clin. Med.*, 50: 78, 1957.

50. Smith, L. H. and Baker, F. A. Pyrimidine metabolism in man. I. The biosynthesis of orotic acid. *J. Clin. Invest.*, 38: 798, 1959.
51. Dzialoszynski, L. M. The clinical value of arylsulfatase estimation in urine. *Clin. chim. acta*, 2: 542, 547, 1957.
52. Code, C. F. The source in blood of the histamine-like component. *J. Physiol.*, 90: 349, 1937.
53. Code, C. F. The histamine-like activity of white blood cells. *J. Physiol.*, 90: 485, 1937.
54. Thiersch, J. B. Histamine and histaminase in chronic myeloid leukemia of man. *Australian J. Exper. Biol. & M. Sc.*, 25: 73, 1947.
55. Valentine, W. N., Pearce, M. L. and Lawrence, J. S. Studies on the histamine content of blood with special reference to leukemia, leukemoid reactions and leukocytosis. *Blood*, 5: 623, 1950.
56. Ehrich, W. E. Histamine in mast cells. *Science*, 118: 603, 1953.
57. Graham, H. T., Wheelwright, F., Parish, H. H., Jr., Marks, A. R. and Lowry, O. H. Distribution of histamine among blood elements. *Fed. Proc.*, 11: 350, 1952.
58. Valentine, W. N., Lawrence, J. S., Pearce, M. L. and Beck, W. S. The relationship of the basophil to blood histamine in man. *Blood*, 10: 154, 1955.
59. Riley, J. F. Histamine in tissue mast cells. *Science*, 118: 332, 1953.
60. Riley, J. F. Editorial. Heparin, histamine and mast cells. *Blood*, 9: 1123, 1954.
61. Hardin, E. B., Valentine, W. N., Follette, J. H. and Lawrence, J. S. Studies on the sulfhydryl content of human leukocytes and erythrocytes. *Am. J. M. Sc.*, 228: 73, 1954.
62. Green, R. and Martin, S. P. The non-protein soluble sulfhydryl content of blood in dyscrasias. *J. Lab. & Clin. Med.*, 45: 119, 1955.
63. Contopoulos, A. N. and Anderson, H. H. Sulfhydryl content of blood in dyscrasias. *J. Lab. & Clin. Med.*, 36: 929, 1950.
64. Weisberger, A. S. and Levine, B. Incorporation of radioactive L-cysteine by normal and leukemic leukocytes in vivo. *Blood*, 9: 1082, 1954.
65. Weisberger, A. S. and Levine, B. Incorporation of radioactive L-cysteine and L-methionine by leukemic leukocytes in vitro. *Blood*, 9: 1095, 1952.
66. Weisberger, A. S. and Levine, B. Some structural requirements for the prevention of leukopenia induced by nitrogen mustard. *J. Clin. Invest.*, 31: 217, 1952.
67. Wagner, R. The estimation of glycogen in whole blood and white blood cells. *Arch. Biochem.*, 11: 249, 1946.
68. Wagner, R. Studies on the physiology of the white blood cell: the glycogen content of leukocytes in leukemia and polycythemia. *Blood*, 2: 235, 1947.
69. Valentine, W. N., Follette, J. H. and Lawrence, J. S. The glycogen content of human leukocytes in health and in various disease states. *J. Clin. Invest.*, 32: 251, 1953.
70. Wislocki, G. B., Rheingold, J. J. and Dempsey, E. W. The occurrence of the periodic acid-Schiff reaction in various normal cells of blood and connective tissue. *Blood*, 4: 562, 1949.
71. Gibb, R. P. and Stowell, R. E. Glycogen in human blood cells. *Blood*, 4: 569, 1949.
72. Wachstein, M. The distribution of histochemically demonstrable glycogen in human blood and bone marrow cells. *Blood*, 4: 54, 1949.
73. Astaldi, G. and Verga, L. The glycogen content of the cells of lymphatic leukemia. *Acta haemat.*, 17: 129, 1957.
74. Mitus, W. J., Bergna, L. J., Mednicoff, I. B. and Dameshek, W. Cytochemical studies of glycogen content of lymphocytes in lymphocytic proliferations. *Blood*, 13: 748, 1958.
75. Meyer, K. H. The chemistry of glycogen. *Advances in Enzymology*, 3: 109, 1943.
76. Follette, J. H., Valentine, W. N., Hardin, E. B. and Lawrence, J. S. Studies on the glucuronic acid content of leukocytes in health and in disease. *J. Lab. & Clin. Med.*, 43: 134, 1954.
77. Martin, von H. and Roka, L. Zur Frage des Heparin-Gehaltes der Blutmastzellen des Menschen. *Acta haemat.*, 10: 26, 1953.
78. Vallee, B. L. Biochemistry, physiology, and pathology of zinc. *Physiol. Rev.*, 39: 443, 1959.
79. Vallee, B. L. and Gibson, J. G., II. Zinc content of whole blood, plasma, leukocytes and erythrocytes. *J. Biol. Chem.*, 176: 445, 1948.
80. Hoch, F. L. and Vallee, B. L. Extraction of zinc-containing protein from human leukocytes. *J. Biol. Chem.*, 195: 531, 1952.
81. Vallee, B. L., Lewis, H. D., Altschule, M. D. and Gibson, J. G., II. Soluble zinc-containing protein extracted from human leukocytes. *Arch. Biochem.*, 48: 347, 1954.
82. Wolff, H. Untersuchungen zur Pathophysiologie des Zinkstoffwechsels. *Klin. Wchnschr.*, 34: 409, 1956.
83. Gibson, J. G., II., Vallee, B. L., Fluharty, R. G. and Nelson, J. E. Studies of the zinc content of the leukocytes in myelogenous leukemia. *Acta contra cancerum*, 6: 1102, 1950.
84. Mathies, J. C. Preparation and properties of highly purified alkaline phosphatase from swine kidneys. *J. Biol. Chem.*, 233: 1121, 1958.
85. Trubowitz, S., Feldman, D., Benante, C. and Kirman, D. Metal requirements of alkaline phosphatases of human and rabbit leukocytes. *Proc. Soc. Exper. Biol. & Med.*, 95: 35–38, 1957.
86. Valentine, W. N., Tanaka, K. R. and Fredricks, R. E. Studies on leukocyte alkaline phosphatase. Role of zinc and magnesium. *J. Lab. & Clin. Med.*, 55: 303, 1960.
87. Nour-Eldin, F. and Wilkinson, J. F. Amino acid content of white blood cells in human leukemias. *Brit. J. Haemat.*, 1: 358, 1955.
88. Iyer, G. Y. N. Free amino acids in leukocytes from normal and leukemic subjects. *J. Lab. & Clin. Med.*, 54: 229, 1959.
89. Rouser, G. Free or easily extractable amino acids in blood cells and body fluids. In: The Leukemias. Etiology, Pathophysiology and Treatment, p. 361. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press, Inc.

The Physiology of Granulocytic Cells in Normal and Leukemic States*

C. G. Craddock, Jr., M.D.

Los Angeles, California

Over the last several years a picture of granulocyte production, distribution and turnover has emerged which, although incomplete, has become much clearer and has drawn together many apparently conflicting observations. The technical obstacles in the path of this type of study are many and explain the great lag in knowledge in this field. Problems of procurement of pure collections of granulocytes, which are normal morphologically and functionally and representative of the cells normally circulating, are too familiar to require elaboration. Nevertheless, certain features of granulocyte kinetics have been established as a basis for the concepts developed here.

Marrow Production. In normal adult man, granulocyte production takes place by the division of immature precursors confined to the marrow space. These precursors include stem cells, myeloblasts, promyelocytes and some myelocytes. The mitotic coefficient (the number of cells entering division at any of these levels) varies, the younger cells showing a higher figure [*1*]. Thus, not all cells capable of division do so under normal conditions, the remaining cells either mature to granulocytes or die at some intervening point. For the myeloblast, about 1 cell per 100 myeloblasts will divide. The cells which are destined to divide probably do so several times while at the myeloblast stage, and then several more times after maturing to promyelocyte and myelocyte levels. Every cell which is capable of maturation will eventually die. However, the earliest progenitor of a cell species will stay at the stem cell level to divide continuously without maturation. Attempts at quantitative expression of myelopoiesis have been dealt with in detailed fashion by Osgood [*2,3*], Rigas [*4*], Patt [*5,6*], Bond et al. [*7*] and Craddock et al. [*8*]. Osgood [*9,10*] has presented cogent arguments favoring a self-regulatory control of proliferation by means of inhibitors of cell division which develop in maturing cells of a given species.

It is of interest to consider the possible mechanisms by which acceleration in the production of granulocytes could occur. The differentiation of more stem cells or pluripotential precursors of all blood cells into myeloblasts would be one important mechanism. A slight increase in mitotic coefficient at the blast cell level would greatly augment the number of cells produced per unit time. A decrease in the time spent in mitosis and the intermitotic interval would accomplish the same thing. A fourth mechanism would be by diminution in the number of cell deaths at various levels before complete maturation. As Stohlman [*11*] has pointed out in regard to erythropoiesis, this phenomenon of "premature" death may be of considerable magnitude in normal physiology.

Alpen and Cranmore [*12*], in their excellent study of the mechanisms of accelerated erythropoiesis in response to blood loss in dogs, could explain their findings only on the basis of more differentiation of stem cells into erythroblasts. It is considered most likely that erythropoietin (the erythropoietic hormone) increases red cell production by acting at this earliest level.

A fundamental difference between the kinetics of erythropoiesis and granulocytopoiesis is the fact that the product of the former is a long-lived cell, whereas the normal granulocyte is produced to be quickly expended. This difference will be discussed in more detail later. It is significant that the red cells resulting from abnormal erythropoietic states are characteristically shorter-lived than normal red cells;

* From the Department of Medicine, School of Medicine, University of California at Los Angeles, and the Hematology Research Laboratory, Wadsworth Hospital, Veterans Administration Medical Center, Los Angeles, California. This work was supported in part by the U. S. Public Health Service, under contract No. H-1069, and by grants from Parke, Davis & Co. and the American Cancer Society.

there is no known condition in which red cells survive longer than in the normal. In the case of the granulocyte, if the normal mature population of non-proliferating cells is replaced by immature cells, some of which are dividing, then the white cells in the periphery survive longer than normal granulocytes. If this is carried to an extreme, as in the blastic leukemias in which the bulk of the population consists of blastic or stem cells, the failure of cells to be expended becomes a factor of major importance. It will be readily apparent that the end result of encroachment of malignant cells will occur even though the malignant tissue is proliferating more slowly than related normal tissues.

Schneiberg [*13*] has studied the phenomena of cell proliferation in man, employing serial marrow aspirations during the acute leukopenic and leukocytic response to bacterial endotoxin. By carrying out quantitative cell counts and mitotic determinations with demecolcin, he could show an almost immediate increase in mitotic coefficient and maturation rate for promyelocytes and myelocytes and, later, an increased granulocyte differentiation at the stem cell level. It is evident that alterations in the mitotic process and hence in the rate of cell production may occur with startling rapidity in response to peripheral stimuli affecting granulocytic tissue.

Granulocyte Maturation. Studies from several different laboratories employing DNA-labeling technics [*6,7,14–16*] have indicated that the *products* of this type of division (i.e., myelocytes which are not dividing, metamyelocytes, stab forms and polymorphonuclears) are normally retained in the marrow cavity for four to six days, with an average of five days, while maturation occurs from the myelocyte level to that of the mature polymorphonuclear cell. Although the interpretation of this phenomenon has differed, it now seems clear that this period of intramedullary maturation comprises a large portion of the total "life span" of the normal granulocytic cell. The cells which are contained within this maturation pool are released in an orderly fashion into the circulating blood.

It is now well established that this intramedullary reserve of mature and maturing granulocytes fulfills an important role as a reserve supply of cells which can be released immediately upon demand [*8,17*]. This simple relationship of marrow granulocytes, which seems so evident in retrospect, was long overlooked or neglected. Failure to take cognizance of this central location of the bulk of mature and maturing granulocytes has beclouded many experiments designed to study the "life span" or turnover of granulocytes in the peripheral blood. When a label is given intravenously with the purpose of labeling circulating granulocytes, labeling of the marrow pool also occurs, to a greater or lesser degree. Since some of the label may attach to dividing cells, the subsequent disappearance of the label from the blood reflects a combination of peripheral turnover, release from the marrow reserve, and dilution of the label by proliferation.

The Circulation of Granulocytes. The cells which pour into the blood in response to an infection or similar pyogenic stimulus do indeed come from the marrow and not from some postulated extramedullary and extravascular pool. This has been shown by experimental creation of a pyogenic stimulus at a time when cells can be followed from the marrow into the blood by isotopic labeling technics [*8*]. It is true that transient alterations in circulating granulocyte numbers may occur as a result of alterations in the vascular dynamics in capillary beds where granulocytes are marginated in large numbers. However, this redistribution of granulocytes between various parts of the vascular system (for example, the increment in central blood granulocyte concentration due to contraction of the pulmonary capillary bed, with release of marginated cells) [*18*] is not to be equated with an increase in the total number of cells in the vascular system.

The cells circulating in the flowing blood, as determined by white cell count and blood volume, comprise a fraction of the total intravascular population. Marginated granulocytes are not a fixed separate pool but are in a state of dynamic exchange with circulating cells. When normal, viable isologous granulocytes are infused in large numbers (equal to or exceeding the calculated circulating mass), there is either no elevation in cell concentration in the blood or a very transient elevation. When cell damage has occurred in the process of obtaining the granulocytes, leukopenia and an acute febrile reaction will accompany the infusion. If the cells are labeled, they can be detected in the blood stream but not in numbers which would be expected if they were entering a closed system with limits defined by the circulating blood volume. When blood counts and determination of the concentration of labeled cells are carried out at frequent

intervals during and after the period the cells are given, irregular spikes can be observed with a falling concentration of labeled cells for thirty to forty minutes. These fluctuations are not so marked if the cells are given slowly. It would appear that during this period of rapid changes in the circulating number of infused cells an equilibration with marginated cells is occurring. In our experience in normal dogs the total intravascular mass of granulocytes would appear to be between one and four times the circulating granulocyte mass. This compares favorably with calculations based on the data of Rosse and Gurney [*19*] who employed morphological labeling by the cross transfusion of normal subjects and patients with the Pelger-Huet anomaly. The actual number of cells marginated at any one time is highly variable and subject to changes in perfusion rate, cell adhesiveness and factors influencing migration into tissue spaces (e.g., bacterial endotoxins and inflammation).

If cells are removed and labeled with as little trauma as possible and reinfused, as in the work of Mauer and his associates [*20*], they disappear in an exponential manner with a half-life of seven to eight hours. It must be remembered, however, that much of the apparent disappearance soon after the infusion is due to equilibration of circulating and marginated cells, and that some of the labeled granulocytes can be detected for forty-eight hours and longer. These data agree with the findings of Rosse and Gurney [*19*]. In these latter observations no cell trauma incident to a labeling procedure was imposed. A relatively rapid turnover of blood granulocytes is also indicated by calculations of the rate of release of granulocytes from the marrow based upon the size and turnover time of the marrow granulocyte reserve [*8*]. It is of more than philosophical interest that the rate of production of granulocytes based upon these calculations of rapid blood turnover is still well below the daily production rate for erythrocytes.

Peripheral Erythrocyte Mass (70 kg. man, blood volume 5,850 cm.^{3}, red blood cells 5×10^6/mm.^{3} or 5×10^9/cm.^{3} $\times$ 5,850 cm.^{3}) $= 29.25 \times 10^{12}$ red blood cells.

Erythrocyte Survival = 120 days.

Daily Erythrocyte Replacement Rate,

$$\frac{29.25 \times 10^{12}}{120} = 2.44 \times 10^{11} \text{ red blood cells/day}$$

or 3.5×10^9 red blood cells/kg./day.

Peripheral Granulocyte Mass (70 kg. man, blood volume 5,850 cm.^{3}, granulocyte count 5,000/mm.^{3} or 5×10^6/cm.^{3} $\times$ 5,850 cm.^{3}) $= 2.92 \times 10^{10} \times 4$ (marginated cells) $= 11.7 \times 10^{10}$.

Granulocyte Survival (average) = 24 hours.

Granulocyte Replacement Rate, 100 per cent/day or 1.17×10^{11} granulocytes/day or 1.67×10^9 granulocytes/kg./day.

It is obvious that the range of error in calculations of this type is very wide. However, the significance of the comparison of erythrocyte and granulocyte production is that estimates of a rapid peripheral turnover of granulocytes are not inconsistent with the steady state productive capacity for hemic cells.

The Utilization of Normal and Abnormal Granulocytes. Another area of contention with respect to the circulation of the normal granulocyte is whether the cell re-enters the blood after migrating into the tissues. Evidence for this has not been forthcoming, and there is a considerable bulk of data showing that this does not occur in normal conditions. It is necessary to differentiate re-entry of cells from tissue spaces from recirculation of cells marginated in the capillaries of lung or held within the sinusoids and pulp spaces of the spleen. It is also important to restrict this opinion of the lack of re-entry of cells to normal granulocytes and not to immature cells of the granulocytic series, as in leukemia. There is good evidence that red cells and lymphocytes may re-enter the blood via lymphatic channels. Leukemic cells may be proliferating in foci throughout the body and discharging cells into the blood. Furthermore, as will be discussed subsequently, immature granulocytes circulate for longer periods than normally mature granulocytes. After the normal mature granulocyte leaves the vascular system, it performs its functions in the tissues, if called upon to do so. How long the granulocyte survives after leaving the blood would depend upon multiple factors but, insofar as the blood is concerned, the cell is lost once it leaves the capillary.

The most evident function of the granulocyte concerns its role in preventing bacterial invasion. The number normally circulating and harbored in various parts of the vascular bed and in tissue spaces is apparently more than adequate to maintain the defenses against the host of bacteria normally present in the gut and tracheo-

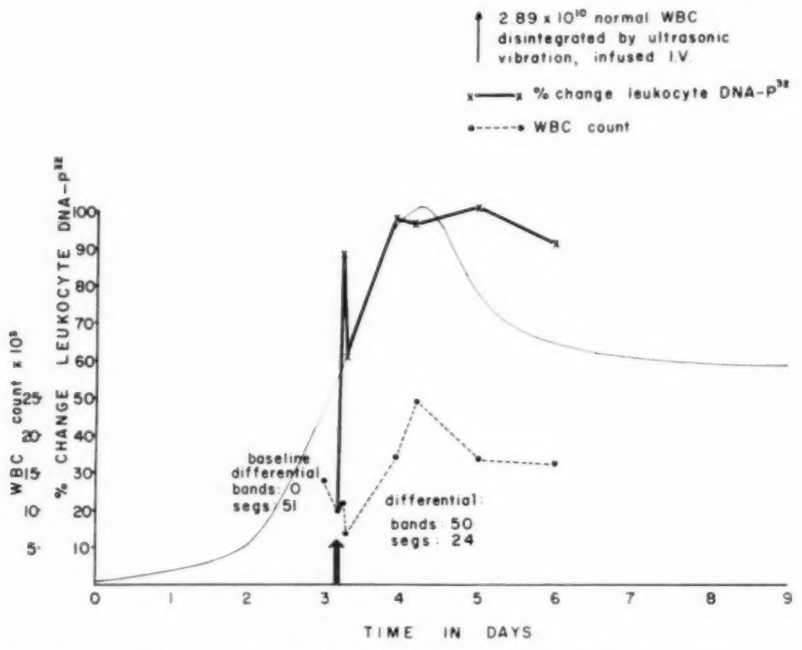

2.89 x 10¹⁰ normal WBC disintegrated by ultrasonic vibration, infused I.V.
x——x % change leukocyte DNA-P³²
•------• WBC count
WBC count x 10³
% CHANGE LEUKOCYTE DNA-P³²
baseline differential bands: 0 segs: 51
differential: bands 50 segs: 24
TIME IN DAYS

FIG. 1. The rapid release of DNA-P^{32}-labeled granulocytes from the marrow as part of the response to intravenous disintegrated homologous granulocytes obtained by leukopheresis. Note the left shift and the sustained plateau of DNA labeling.

bronchial tree. A decrease in granulocyte number or the replacement of functionally adequate, mature granulocytes with immature abnormal varieties, as in the leukemias, permits overthrow of this important homeostatic mechanism. Under normal conditions the mature granulocyte is probably consumed quickly. When bacterial invasion threatens, the movement of granulocytes into the inflamed area accelerates, and this is compensated by acceleration of release of cells from the marrow granulocyte reserve. This arrangement permits a large number of mature cells to be called forth. Although definitive studies in this regard are lacking, it is not unreasonable to suppose that the mature polymorphonuclear neutrophil has more of the equipment necessary for phagocytosis and digestion of bacteria than its immature precursor, and that the period of intramedullary maturation is important for this reason.

There are many reasons to believe that the normal mature granulocyte is destined for rapid expenditure after it leaves the marrow. Immature granulocytes, however, may remain in the circulation longer, in average terms. If an animal is subjected to leukopheresis there is first an acceleration in the entry of cells from the marrow, and the granulocyte concentration in the blood rises rapidly after the procedure is terminated. As the blood granulocyte level plateaus, the rate of release slows below normal, but the leukocytosis persists for twenty-four to forty-eight hours. This suggests that the younger forms released in response to leukopheresis remain in the circulation longer than fully mature granulocytes. This point is further supported by the type of data shown in Figure 1. Here a marked leukocytosis was induced, with rapid release of "new cells" from the labeled marrow. There was considerable "left shift." The fall in peripheral blood granulocyte DNA-P^{32} specific activity is less rapid than normal, indicating that these immature cells circulate for a longer time, on the average, than the more mature population in the normal steady state.

Additional evidence supporting this rather unorthodox concept of more prolonged circulation of immature cells derives from the fact that infections in man or experimental situations which are known to remove cells rapidly from the peripheral blood fail to cause a rapid fall in the curve of disappearance of DNA-labeled cells in patients with chronic granulocytic leukemia or myeloid metaplasia in whom a larger proportion of the circulating cells are immature. The data suggest that the period of time spent by the

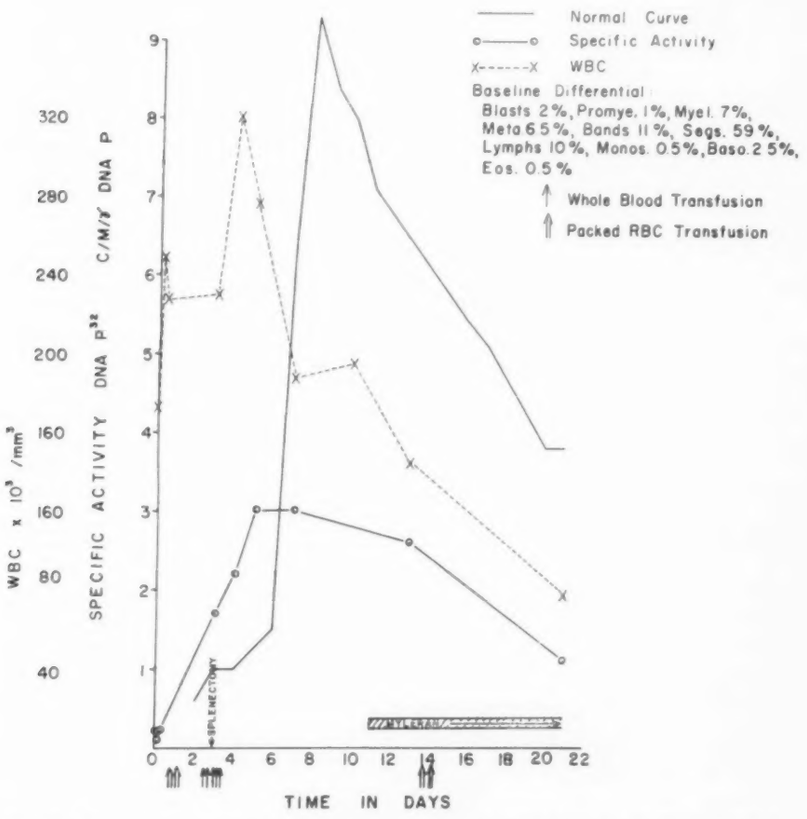


FIG. 2. The appearance and disappearance of DNA-P^{32}-labeled cells in the peripheral blood of a patient with myeloid metaplasia of the spleen and liver. Splenectomy on day 3 did not prevent the continued rise of the curve and did not result in a rapid disappearance in labeled cells.

granulocyte in the peripheral blood is at least as short in the normal steady state as in conditions leading to accelerated granulocytopoiesis.

We have observed a patient with splenic myeloid metaplasia of advanced degree in whom removal of the spleen during the course of the DNA-labeling curve failed to alter the curve. (Fig. 2.) Despite surgical removal of an enormous mass of granulocytopoietic and erythropoietic tissue and massive loss of blood, there was no fall in the white cell DNA specific activity. This can only mean that those cells (or the products of division of those cells) the DNA of which was labeled soon after the P^{32} was given, remain in the body for a long period of time in comparison with a normal mature granulocyte population. The data in Figure 3 show similar findings in a patient with a myelomonocytic type of leukemia. Disappearance of the labeled cells occurred in this instance in association with a pyogenic pneumonia which occurred fifty-four days after labeling.

Figure 4 shows the DNA-labeling curve of a patient with acute myeloblastic leukemia in the terminal blastic phase of the disease resistant to the effect of 6-mercaptopurine and adrenal steroids. Prior to giving the *in vivo* DNA label, *in vitro* studies of the circulating cell population indicated a rapid and marked uptake of P^{32} into DNA. H^3-thymidine radioautography revealed that 12 to 15 per cent of the patient's cells in the peripheral blood incorporated the label, although the grain counts per labeled cells were low after incubation for three hours and twenty-four hours. It would appear that although total DNA synthesis by the leukemic population as a whole was enormous, the rate of DNA synthesis per cell was quite slow. This has been observed in several leukemic states, as will be described subsequently. This degree of DNA synthesis by the circulating cell population is indicative of the great size of the proliferating cell mass in blastic leukemias of this type.

It will be noted that profound fluctuations in

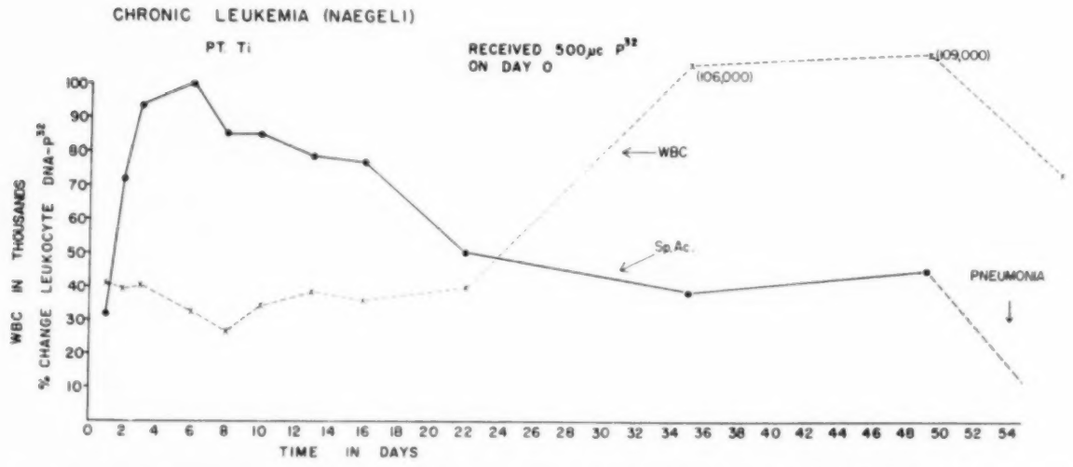


FIG. 3. The rapid appearance and prolonged disappearance of DNA-P^{32}-labeled leukemic cells in a patient with myelomonocytic leukemia. Labeled cells remained in the circulation for a long period and finally disappeared at the time of a fatal pneumonia.

the concentration of blast cells in the peripheral blood, associated with hyperuricemia, resulted from the intravenous administration of normal granulocytes. Unfortunately, the donors had received 0.2 gamma of a purified lipopolysaccharide endotoxin from Salmonella abortus equi (pyrexal, courtesy of The Wander Co.) twenty-four hours prior to phlebotomy. This had been given to stimulate a leukocytosis since, for the purposes of this experiment, it was desirable to obtain a high yield of granulocytes.

The pyrogenic reaction in this case was probably due to immediate release of endogenous leukocyte-endotoxin pyrogen [*21*], since these unlabeled cells failed to circulate, as indicated by the lack of fall in DNA-P^{32} level in the circulating labeled cell population. The mechanisms of the apparent destruction of

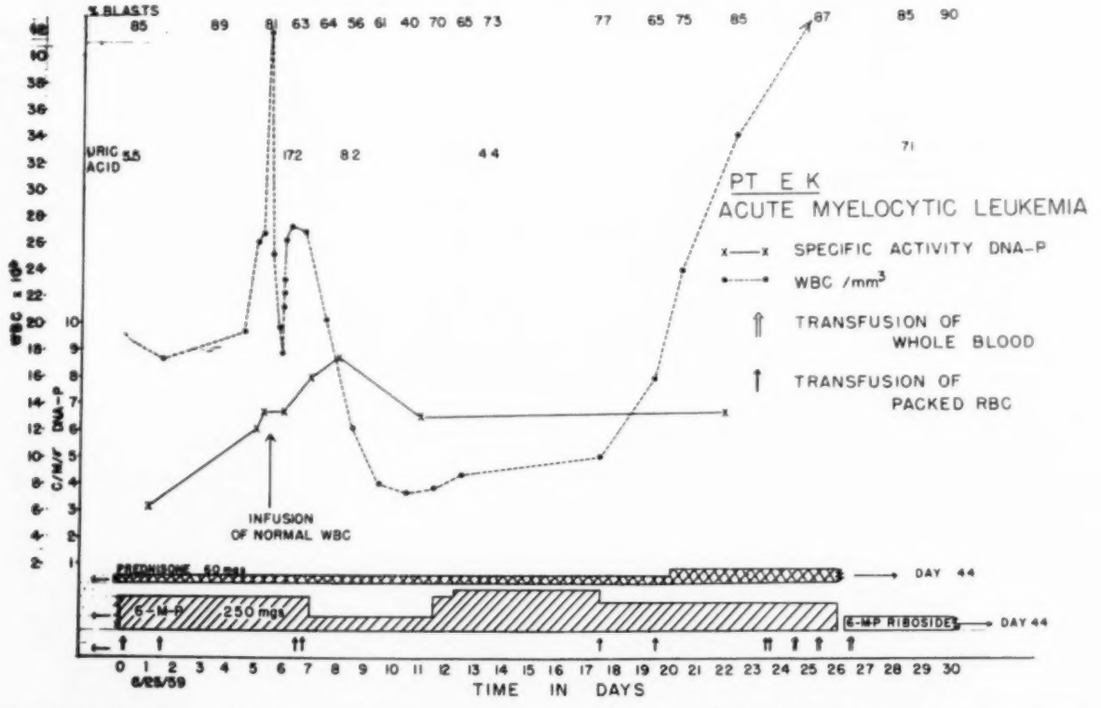


FIG. 4. Marked fluctuation in total circulating cell numbers in a case of myeloblastic leukemia without acute alterations in the extent of DNA-P^{32} labeling in the circulating cell population.

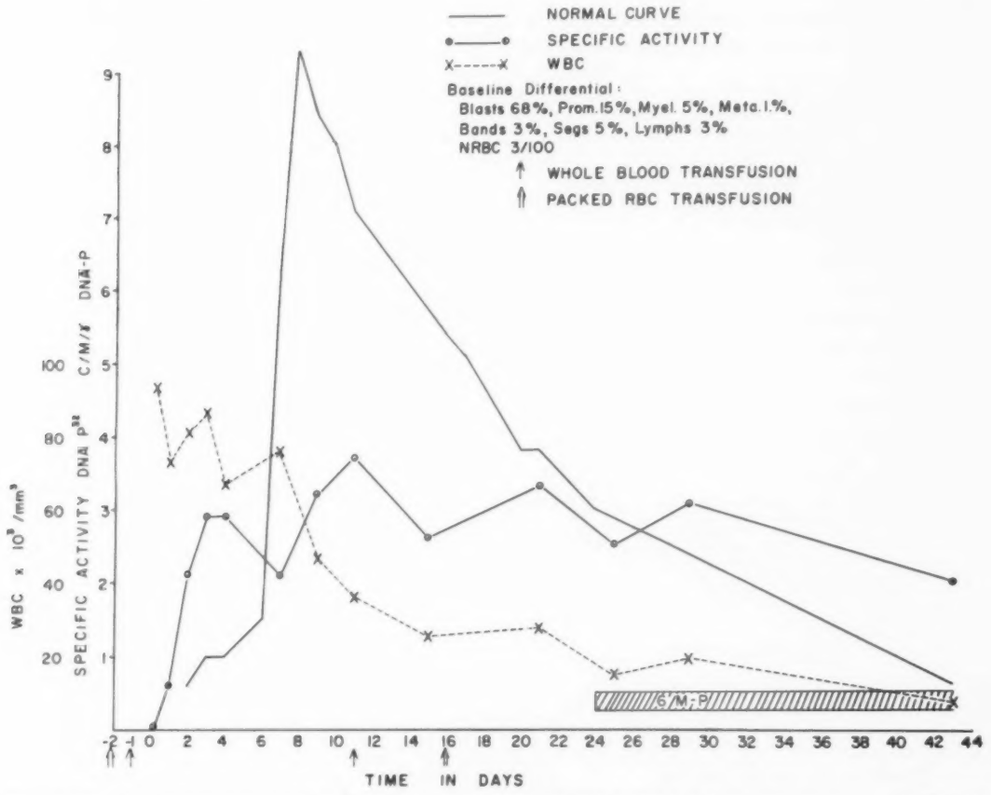


FIG. 5. Rapid appearance and prolonged persistence of DNA-P^{32}-labeled cells in a case of blastic leukemia despite falling cell concentration.

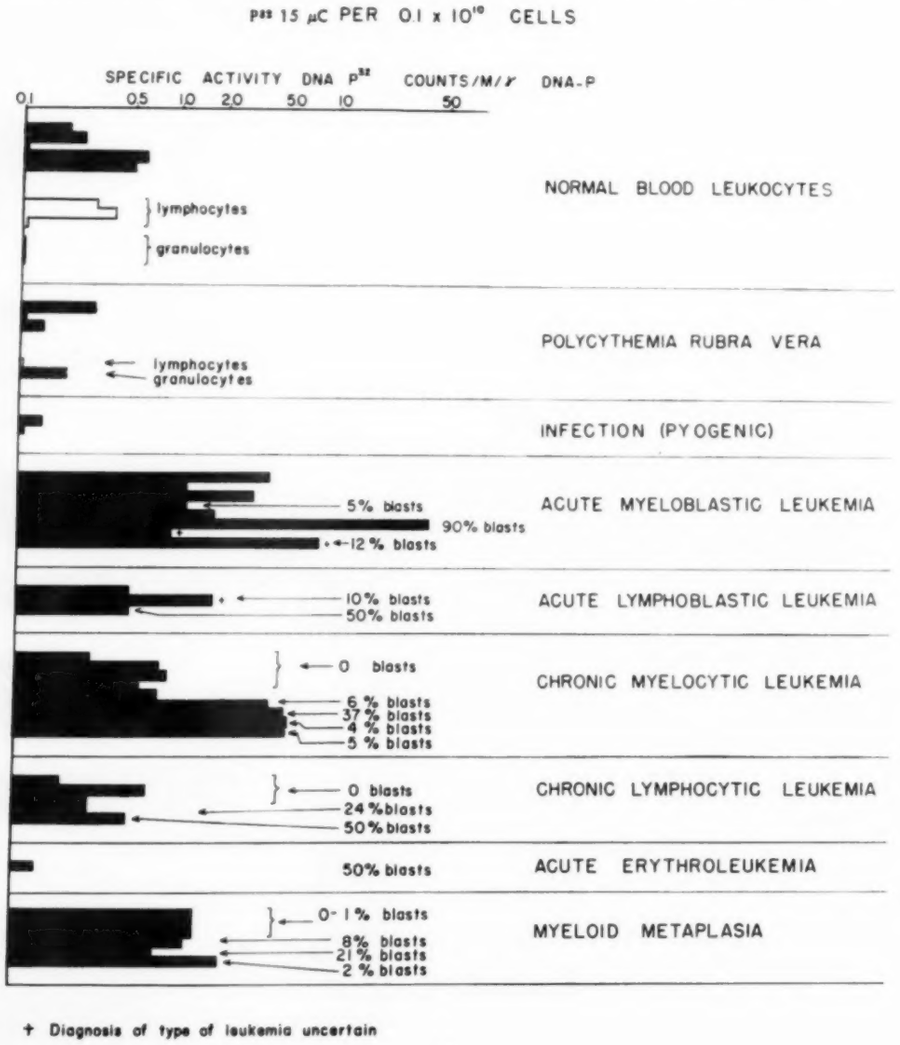
leukemic cells and the temporary improvement in the blood picture are unknown and may be non-specific. The portion of this rather complicated experiment which is germane to this discussion is the stable time-course of the DNA-P^{32} specific activity curve in spite of the extreme fluctuations in cell numbers and ages incident to the cell destruction. It is obvious that the persistence of the DNA label does not depend upon the survival of the labeled cells for long periods when one is dealing with a cell population in which a large percentage of cells are dividing. The course of the DNA-labeling curve in this type of study is a reflection of an increasing ratio of labeled to unlabeled cells (due to the greater loss of unlabeled cells when they comprise the bulk of the cell population), and a decreasing concentration of the DNA label per cell as successive mitoses occur. With repeated cell divisions a point is reached where further dilution of the DNA label is insignificant, and by this time the entire leukemic cell population is labeled. At this point no change in leukocyte DNA-P^{32} specific activity will occur regardless of how many cells are suddenly destroyed.

Figure 5 shows similar data in another patient with acute blastic leukemia. These data indicate the limitations of isotopic labeling of this type in the determination of cell survival in an immature proliferating cell population. It is clear that the same limitations apply to any cell label unless the concentration of the label for each cell is determined. Of course, the situation is different when one is dealing with a non-dividing peripheral blood population. When we speak of "life span" or survival time of blood cells, the terms should be qualified to apply only to the product of cell division which will remain functionally and morphologically intact for a finite period before death, and which is incapable of further division. Leukemic cells, of the blastic or stem cell type, do not maturate into cells which are mature by morphologic, biochemical and functional criteria; they live to divide. The rate of division may be faster or much slower than the normal population; the end result is the same. The important difference between the competing normal and leukemic cell populations is that the products of normal cell division will *all* die (at least in the myeloid cell populations),

whereas a small but steadily increasing percentage of leukemic cells do not die in the usual sense of the word, but divide continuously without maturation. This does not mean, of course, that the number of cells dying and disintegrating per unit time is less in the leukemias than in the normal. Indeed, it is usually much greater in the advanced leukemic state. Nevertheless, the tremendous increase in bulk of proliferative tissue outstrips the accelerated destruction of cells and the population becomes progressively immature. Eventually the bulk of cells are stem or blast cells capable of infinite division as long as they can maintain life.

These comments concerning the difficulty in drawing any conclusions as to turnover rate of a proliferating group of cells from isotopic labeling methods are pertinent to the interpretation of lymphocyte kinetics because cell division of some normal lymphoid cells occurs in the blood and tissues [*8,22,23*]. In this sense, therefore, the proliferating lymphocytes are like leukemic cells and the long persistence of some labeled cells does not necessarily denote a long life span for the mature non-dividing products of these cells.

To return to the normal granulocyte, it is not surprising that cells which must complete their maturation in the peripheral blood instead of in the marrow would circulate longer than the fully mature normal granulocyte. The motility,



6A

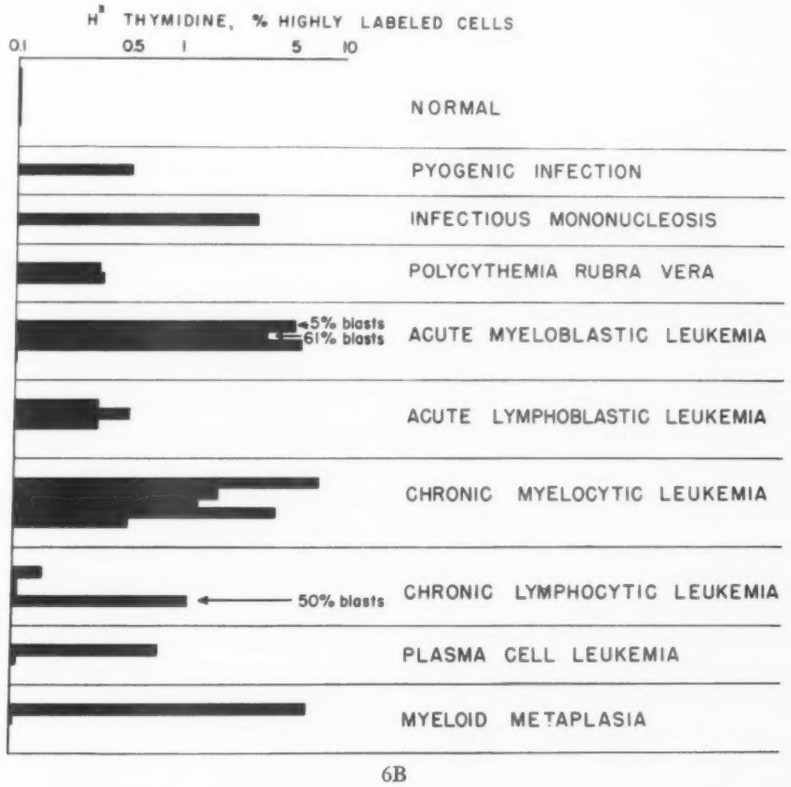
FIG. 6A and B. The *in vitro* incorporation of P^{32} and H^3-thymidine by hemic cells. Cells suspended in buffered Hank's solution, pH 7.4, concentration 10,000/mm.³ Incubations at 37°C. H^3-thymidine specific activity 1.9 C/mM, 0.5 μc./ml. The separation of normal

phagocytic, leukotactic and agglutinative properties of immature cells are probably less than for the normal granulocyte. As Dr. George Cartwright has so aptly expressed it: "perhaps the granulocyte is functionally concerned with death and not survival. It dies in the process of doing its work. It is more or less made to die" [*24*]. Viewed from this aspect, the immature granulocyte which is not ready to die is not a functionally normal cell.

In Vitro DNA Synthesis Rate of Leukemic Cells as a Function of Cell Growth. In the leukemic patient whose disease is progressing and not being held in check by therapeutic measures the vascular system and tissues may be flooded with immature leukemic cells. Some of these cells are proliferating as they circulate, and are synthesizing DNA, as indicated by *in vitro* labeling of this material in short-term cultures. This growth of leukemic blood leukocytes has been the subject of recent study [*23,25*]. The systems we have used consisted of suspensions of leukocytes or whole blood in buffered Hank's solution-serum or lymph mixtures, the leukocyte concentrations adjusted to approximately 10,000/mm.^{3} and the addition of H^3-thymidine, 0.25 to 1.5 μc./ml., and P^{32}, 15 μc./10^9 cells. Incubation times for both isotopes were three and twenty-four hours. Subsequently, it has become clear that a more precise estimate of DNA synthesis rate can be obtained by sampling at frequent intervals (every half hour for three hours).

The results of three-hour incubations of leukemic cells of different morphologic varieties with the two isotopes are shown in Figure 6. In these data the term per cent cells labeled with H^3-thymidine includes only cells with heavy labeling (grain counts in excess of 15) unless otherwise specified. It will be noted that the results of the two methods of DNA labeling agree in general terms, and it has become our practice to employ both methods as checks against each other.

Several points are evident from these rather preliminary data. First, the extent of DNA labeling under these conditions does not correlate with the degree of morphologic immaturity of the cell population. Thus, in the myeloprolif-



6B

blood granulocytes and lymphocytes is indicated in A. It will be noted that the cells synthesizing DNA are in the "lymphocyte" fraction. This includes all types of mononuclear cells.

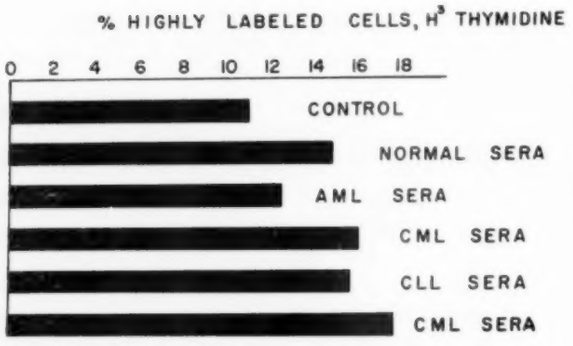


FIG. 7. The effect of various serums on dog thoracic duct lymphocyte DNA labeling.

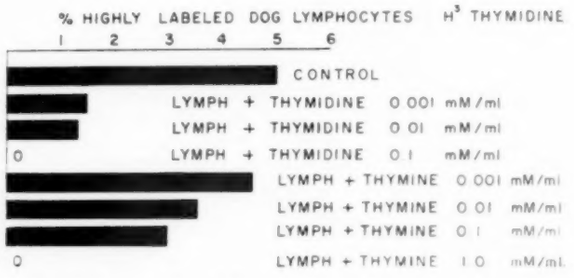

% HIGHLY LABELED DOG LYMPHOCYTES H³ THYMIDINE
1 2 3 4 5 6
CONTROL
LYMPH + THYMIDINE 0.001 mM/ml
LYMPH + THYMIDINE 0.01 mM/ml
0
LYMPH + THYMIDINE 0.1 mM/ml
LYMPH + THYMINE 0.001 mM/ml
LYMPH + THYMINE 0.01 mM/ml
LYMPH + THYMINE 0.1 mM/ml
0
LYMPH + THYMINE 1.0 mM/ml

FIG. 8. Effect of thymidine and thymine on uptake of H^3 thymidine by dog lymphocytes in lymph (3 hour incubation with thymidine 0.5 μc./ml. lymph lymphocyte concentration 10,000/mm³. Specific activity H^3 thymidine 1.9 C/mM).

erative disorders the highest degree of labeling was often observed in instances with a low percentage of myeloblasts. Second, those conditions which were clearly lymphocytic, whether chronic or acute, showed low uptake. This is of especial interest in view of the high degree of labeling observed in normal thoracic duct lymphocytes and in the atypical lymphocytes of infectious mononucleosis. Two patients with plasma cell leukemia with large numbers of blast cells in the blood showed low DNA labeling.

If it is true that DNA synthesis occurs in proliferating cells only, and if it is assumed that this phenomenon correlates with the rate of cell division and intermitotic interval, it would appear that blastic leukemias are not always associated with an acceleration in mitotic rate. Indeed, the mitotic cycle of these leukemic blasts may be much longer than for the normal blood cell precursors, as shown by Lajtha [*26*]. The total bulk of proliferating tissue in relationship to the bulk of maturing and dying cells is probably more important in the over-all course of the disease. More evidence is needed, however, before it can be stated with assurance that the rate of incorporation of an isotope into DNA is a specific measure of either DNA synthesis rate or mitotic activity. For one thing, the uptake of the label may be obscured by the amount of unlabeled intracellular precursor. For another, cells which synthesize DNA rapidly may not divide subsequently or may do so in a delayed manner. More data correlating mitotic indices with DNA synthesis are needed in this regard.

We have studied the effect of different types of serums and plasmas in the incubation mixtures of immature cells, and it is clear that they differ in their influences on DNA synthesis, but no consistent pattern emerges. Figure 7 shows an experiment employing dog thoracic duct lymphocytes, with little variation in the percentage of labeled cells. It has long been known that serums differ in their influence on cell growth in tissue culture [*10*]. It has also been shown that serums differ in their influence on nucleic acid synthesis [*27–29*], and it has been postulated that this may be due to an inhibitor(s) which is more apparent in normal serum than in that of leukemic patients [*29*]. The possibility exists that this effect is due to differences in serum content of DNA precursors. The sensitivity of H^3-thymidine labeling of cell DNA to the amount of carrier thymidine is shown in Figure 8.

Dog thoracic duct lymphocytes provide a source of proliferating cells of excellent purity and morphologic quality for this type of study. There is no cell trauma incident to obtaining and separating the cells for study and a significant percentage of the cells (4 to 15 per cent) are actively synthesizing DNA. The lymph also provides an excellent culture medium for incubation of the cells, a system which reproduces the *in vivo* conditions very closely for lymphocytes. As part of the study of DNA synthesis in these cells, we have observed a peculiar effect of granulocytes. Mature canine or human normal granulocytes markedly inhibit the uptake of H^3-thymidine and P^{32} into DNA by lymphocytes when the two types of cells are present in the same incubation system. This inhibition is marked, as shown in Figure 9, and in some instances totally abolished uptake of the label after three hours incubation. During and following such an incubation both varieties of cells appeared normal morphologically on stained smears and on phase microscopy. Vital staining characteristics and motility were not altered.

The ratio of granulocytes to lymphocytes in

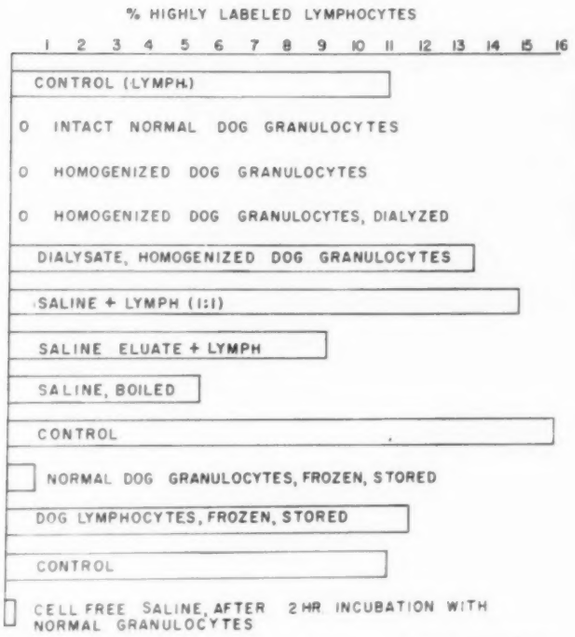

% HIGHLY LABELED LYMPHOCYTES
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
CONTROL (LYMPH.)
0 INTACT NORMAL DOG GRANULOCYTES
0 HOMOGENIZED DOG GRANULOCYTES
0 HOMOGENIZED DOG GRANULOCYTES, DIALYZED
DIALYSATE, HOMOGENIZED DOG GRANULOCYTES
SALINE + LYMPH (1:1)
SALINE ELUATE + LYMPH
SALINE, BOILED
CONTROL
NORMAL DOG GRANULOCYTES, FROZEN, STORED
DOG LYMPHOCYTES, FROZEN, STORED
CONTROL
CELL FREE SALINE, AFTER 2 HR. INCUBATION WITH NORMAL GRANULOCYTES

FIG. 9. Dog thoracic duct lymphocytes inhibition of DNA labeling with H^3 thymidine by granulocytes (3 hour incubation).

these experiments was 1:1. Figure 10 shows the inhibitory effect with lesser concentrations of granulocytes. Experiments are under way to determine the minimum concentration of granulocytes which will effect the phenomenon. The granulocytes in these experiments were obtained by dextran sedimentation of peripheral blood from the same dog whose lymphocytes were being studied. In other instances granulocytes obtained by leukopheresis were employed. The inhibition was equally striking with viable, actively motile granulocytes and cells disintegrated by homogenization, ultrasonic vibration or freezing and thawing. Some inhibitory effect could be shown in saline extracts of such cells, but not as much as in the cell fragments. The inhibitory effect was not removed by dialysis overnight against water and did not appear in the dialysate. The inhibitor effect was found in the supernatant saline and Hank's solution following incubation of a high concentration of granulocytes for two hours at 37°c. These cells were undamaged as much as possible after this type of handling and were 90 per cent viable by the criteria of vital staining characteristics and motility. The inhibitory effect may be a secretory product of the granulocyte.

Normal human granulocytes, intact or disintegrated, also inhibit the uptake of label

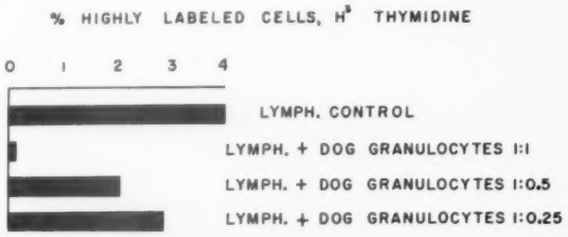

% HIGHLY LABELED CELLS, H³ THYMIDINE
0 1 2 3 4
LYMPH. CONTROL
LYMPH. + DOG GRANULOCYTES 1:1
LYMPH. + DOG GRANULOCYTES 1:0.5
LYMPH. + DOG GRANULOCYTES 1:0.25

FIG. 10. Inhibition of DNA labeling of dog thoracic duct lymphocytes by varying concentrations of granulocytes.

into the DNA of dog lymphocytes. (Fig. 11.) Dog lymphocytes and erythrocytes do not inhibit the phenomenon, whether intact or disintegrated. Cells from one patient with acute myelocytic leukemia and one patient each with chronic granulocytic leukemia and chronic lymphocytic leukemia in relapse fail to inhibit DNA synthesis by dog thoracic duct lymphocytes as much as normal cells. (Fig. 12.) This was true with the intact cells and with disintegrated cells. The inhibitor effect of normal human granulocytes on leukemic cells is a subject of present study, although sufficient data have not been obtained to permit any conclusion. It is not known at present whether this inhibitory effect of granulocytes occurs with proliferating populations of normal erythroid cells. Preliminary studies of marrow showing granulocytic hyper-

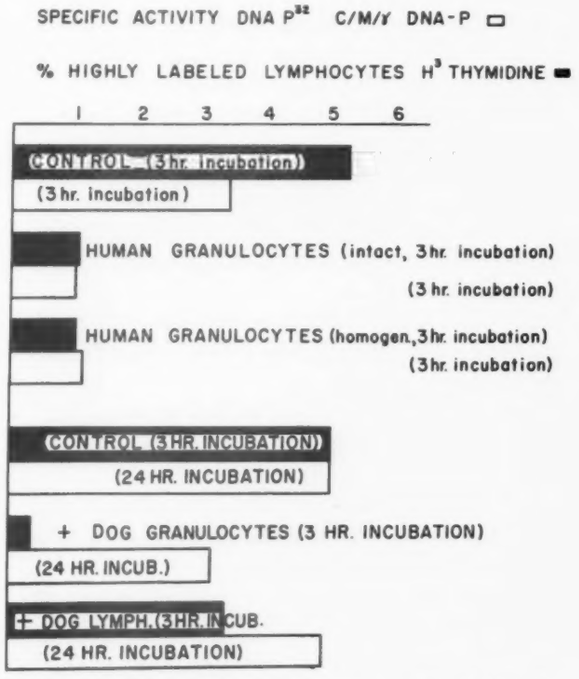

SPECIFIC ACTIVITY DNA P³² C/M/γ DNA-P
% HIGHLY LABELED LYMPHOCYTES H³ THYMIDINE
1 2 3 4 5 6
CONTROL (3hr. incubation)
(3 hr. incubation)
HUMAN GRANULOCYTES (intact, 3hr. incubation)
(3 hr. incubation)
HUMAN GRANULOCYTES (homogen, 3hr. incubation)
(3hr. incubation)
CONTROL (3 HR. INCUBATION)
(24 HR. INCUBATION)
+ DOG GRANULOCYTES (3 HR. INCUBATION)
(24 HR. INCUB.)
+ DOG LYMPH. (3HR. INCUB.
(24 HR. INCUBATION)

FIG. 11. Granulocyte inhibition of thoracic duct lymphocyte DNA labeling with P^{32} and H^3 thymidine.

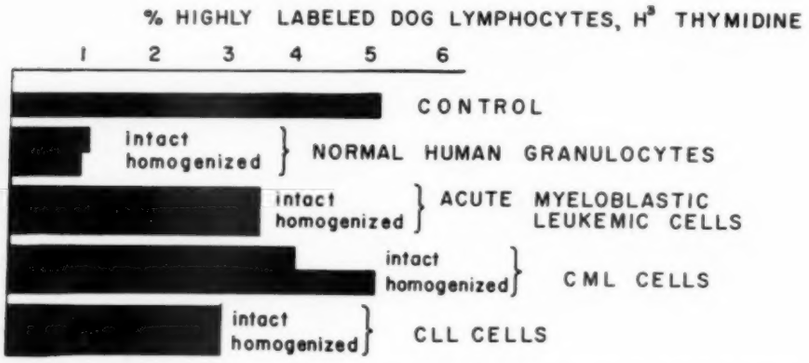


FIG. 12. Effect of various types of leukemic cells on DNA labeling of dog thoracic duct lymphocytes (3 hour incubation with H^3 thymidine, 0.5 μc./ml. lymph lymphocyte concentration 10,000/mm³).

plasia and immaturity indicate that this inhibitor effect of mature granulocytes influences the rate of DNA synthesis of granulocytic tissue. The chemical properties of the inhibitor effect are under study.

The presence of a potent inhibitor of DNA synthesis in mature normal granulocytes would be consistent with the postulated "feed-back" control system of hemopoiesis developed by Osgood [*9,10*]. It would also help explain many observations relative to the influence of various serums on cell growth and the occasional striking suppression of the leukemic process by blood transfusion [*30,31*]. The rapidity with which the hematologic changes may occur after the transfusion in such cases is not inconsistent with the leukopenic effect being one of suppression of DNA synthesis and cell division, since the non-dividing granulocytic population has a brief life span. The observations by Bierman et al. [*32*] concerning the therapeutic effect of white cell products in acute lymphoblastic leukemia are pertinent to these data. Such a mechanism would also elucidate the results of leukopheresis.

It has been pointed out previously that the removal of cells peripherally accelerates the release of granulocytes from the marrow reserve and promotes an increase in mitotic activity. Conversely, the intravenous infusion of large numbers of mature granulocytes in dogs inhibits the release of cells from the marrow, as shown in Figure 13. Here the normal unlabeled cells obtained by leukopheresis were infused at a time when the cells with labeled DNA would normally have entered the blood from the marrow. It will be observed that the appearance of labeled cells was delayed for about twenty-four hours, and that the slope of the subsequent increment in new cells was similar to that in the steady-state animal. If new cells continued to enter at the same rate as normal during and after the infusion and the delay was due entirely to persistence of the infused unlabeled cells in the circulation (and hence a larger unlabeled pool to be replaced by labeled cells), the slope would be less steep tnan normal. It would appear that the entry of labeled cells from the marrow reserve was temporarily stopped by the infusion, to be resumed at the same rate after the excess cells in the periphery had been eliminated. This interpretation is predicated on the assumption that the infused cells function and survive normally, an assumption which is difficult to establish. Therefore, the data suggest but do not prove that granulocytopoiesis is suppressed by hypertransfusion of granulocytes in a manner similar to the effect of red cell transfusion on erythropoiesis. It is hoped that correlation of this type of data with alterations in mitotic activity will provide information as to how this suppression of cell production comes about.

If disintegrated granulocytes are administered intravenously, a severe reaction ensues, as described by Weisberger [*33*]. In dogs this is characterized by fever, leukopenia followed by leukocytosis, intravascular clotting and defibrination, followed by a hemorrhagic state, convulsions and tachycardia. Much of the reaction is related to the release of thromboplastic and pyrogenic substances. The reaction is associated with a massive release of granulocytes from the marrow as shown in Figure 1, and this occurs even though the cell fragments are administered via the splenic artery in an attempt to offset the systemic toxicity. This severe reac-

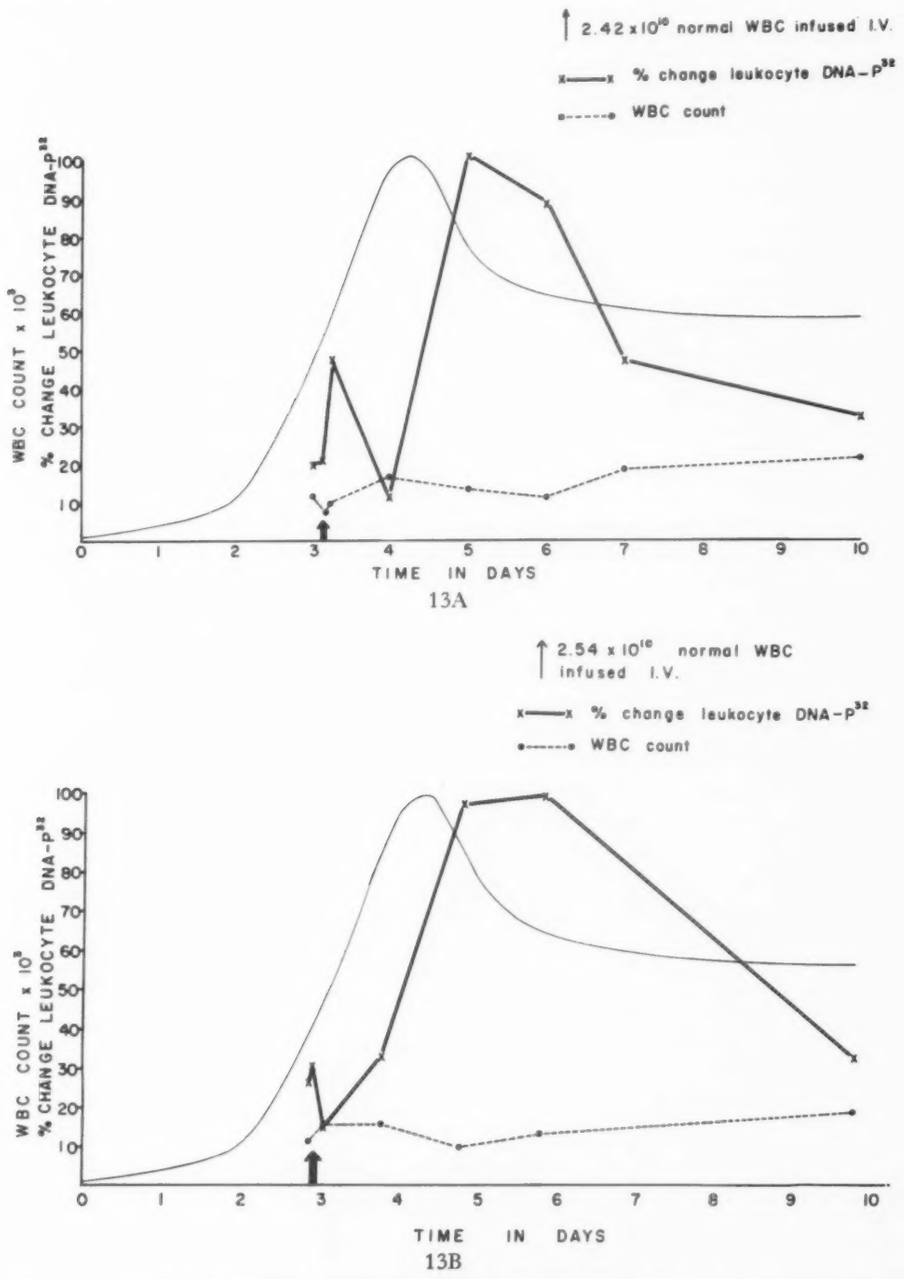


FIG. 13. A and B, inhibition of the release of DNA-labeled granulocytes from the marrow by the intravenous infusion of normal homologous unlabeled granulocytes obtained by leukopheresis. The solid fine line represents the normal time of appearance of DNA-labeled cells in the steady state.

tion, which is probably non-specific, prevents the demonstration of *in vivo* inhibition of granulocytopoiesis by crude products of disintegrated granulocytes. Nevertheless, the evidence for the control of granulocyte release from the marrow being geared in some way to the mature granulocyte is more than suggestive. It is attractive to consider this control mechanism as being related to the inhibitory effect of some product of the mature granulocyte on cell proliferation, as shown in the foregoing experiments with immature lymphocytes.

The preponderance of normal myeloid marrow consists of maturing cells in the marrow

granulocyte reserve. If these more mature cells inhibit the growth rate of precursors, then the removal of mature cells at an accelerated rate, as in response to leukophoresis or infection, will increase the rate of proliferation simply by reducing the inhibitory effect of the mature cells in the environment of their precursors. This hypothesis has the merit of simplicity, although much work must be done to establish its validity. The implications of this "built-in" control of granulocyte production are obvious, and the possible relationship of this system to the leukemic state should afford an area of interesting study for the future in view of the preliminary evidence that leukemic cells are deficient in the inhibitor effect.

These data are one additional bit of information supporting the general concept that the failure of cell maturation in leukemia is associated with a loss of factors which inhibit cell proliferation. It is doubtful that this lack of inhibition would involve identical mechanisms in all leukemias. In one instance the inhibitor effect might be present in the leukemic cell but the material is not released from the cell. In another instance the proliferating population might be resistant to such inhibition even if present. In others, the inhibitors might be blocked in their action or counterbalanced by stimulators of cell growth. In any event, these phenomena warrant intensive further study.

Acknowledgment: This work was performed in collaboration with Drs. Seymour Perry, John S. Lawrence, Leo Buxbaum and George R. Pieper, with the technical assistance of Miss Mary H. Baker, Mrs. Gloria Buchbinder, Mrs. Netty Buras and Mr. Robert Yelenosky.

REFERENCES

1. Journoud, R. Mitose et intereinese cellulaire dans diverses hémopathies. L'interet du coefficient mitotique. Essai sur l'interpretation des leucoses humaines. *Sang*, 24: 640, 1954.
2. Osgood, E. E. Number and distribution of human hemic cells. *Blood*, 9: 1141, 1954.
3. Osgood, E. E. Control of peripheral concentration of leukocytes. In: Symposium on Homeostatic Mechanisms. Brookhaven Symposia in Biology, vol. 10. New York, 1958. Brookhaven National Laboratory.
4. Rigas, D. A. Kinetics of isotope incorporation into the desoxyribonucleic acid (DNA) of tissues; life span and generation time of cells. *Bull. Math. Biophys.*, 20: 33, 1958.
5. Patt, H. M. A consideration of myeloid-erythroid balance in man. *Blood*, 12: 777, 1957.
6. Patt, H. M. and Maloney, M. A. Kinetics of neutrophil balance. In: The Kinetics of Cellular Proliferation, pp. 201–207. Edited by Stohlman, F. New York, 1959. Grune & Stratton.
7. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R. and Robertson, J. S. Cell turnover in blood and blood-forming tissues studied with tritiated thymidine. *Ibid.*, pp. 188–200.
8. Craddock, C. G., Perry, S. and Lawrence, J. S. Control of the steady state proliferation of leukocytes. *Ibid.*, pp. 242–259.
9. Osgood, E. E. A unifying concept of the etiology of the leukemias, lymphomas and cancers. *J. Nat. Cancer Inst.*, 18: 155, 1957.
10. Osgood, E. E. Regulation of cell prolferation. In: The Kinetics of Cellular Proliferation, pp. 282–288. Edited by Stohlman, F. New York, 1959. Grune & Stratton.
11. Stohlman, F. Observations on the kinetics of red cell proliferation. *Ibid.*, pp. 318–324.
12. Alpen, E. L. and Cranmore, D. Observations on the regulation of erythropoiesis and on cellular dynamics by Fe^{59} autoradiography. *Ibid.*, pp. 290–300.
13. Schneiberg, K. A study of granulopoiesis in leukocytosis. *Haemat. Polonica*, 3: 183, 1959.
14 Ottesen, J. On the age of human white cells in the peripheral blood. *Acta physiol. scandinav.*, 32: 75, 1954.
15. Kline, D. L. and Cliffton, E. E. Life span of leukocytes in man. *J. Appl. Physiol.*, 5: 79, 1952.
16. Perry, S., Craddock, C. G. and Lawrence, J. S. Rates of appearance and disappearance of white blood cells in normal and in various disease states. *J. Lab. & Clin. Med.*, 51: 501, 1958.
17. Craddock, C. G., Perry, S. and Lawrence, J. S. The dynamics of leukopoiesis and leukocytosis as studied by leukopheresis and isotopic techniques. *J. Clin. Invest.*, 35: 285, 1956.
18. Bierman, H. R., Kellog, K. H., Cordes, F. L., Byron, R. L., Polhemus, J. A. and Rappaport, S. The release of leukocytes and platelets from the pulmonary circulation after epinephrine. *Blood*, 7: 683, 1952.
19. Rosse, W. F. and Gurney, C. W. The Pelger-Huet anomaly in three families and its use in determining the disappearance of transfused neutrophiles from the peripheral blood. *Blood*, 14: 170, 1959.
20. Mauer, A. M., Athens, J. W., Warner, H. R., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M. An analysis of leukocyte radioactive curves obtained with radioactive diisopropylfluorophosphate (DFP^{32}). In: The Kinetics of Cellular Proliferation, pp. 231–239. Edited by Stohlman, F. New York, 1959. Grune & Stratton.
21. Petersdorf, R. G. and Bennett, I. L., Jr. The experimental approach to the mechanisms of fever. *Arch. Int. Med.*, 103: 991, 1959.
22. Gowans, J. L. The effect of continuous re-infusion of lymph and lymphocytes on the output of lymphocytes from the thoracic duct of unanesthetized rats. *Brit. J. Exper. Path.*, 38: 67, 1959.
23. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., Brecher, G. and Schork, P. K. Proliferative potentials of bone marrow and blood

cells studied by *in vitro* uptake of H^3 thymidine. *Acta haemat.*, 21: 1, 1959.

24. Cartwright, G. E. Discussion of leukocyte production and survival using C^{14} and P^{32}. In: The Kinetics of Cellular Proliferation, p. 183. Edited by Stohlman, F. New York, 1959. Grune & Stratton.
25. Brecher, G. Data presented at Ross Conference on Current Concepts in Leukemia. Sponsored by Ross Laboratories, Los Angeles, California, November 1959.
26. Lajtha, L. G. On DNA labeling in the study of the dynamics of bone marrow cell populations. In: The Kinetics of Cellular Proliferation, pp. 173–182. Edited by Stohlman, F. New York, 1959. Grune & Stratton.
27. Thomas, E. D. and Lochte, H. L., Jr. Desoxyribonucleic acid synthesis by bone marrow cells *in vitro*. *Blood*, 12: 1086, 1957.
28. Thomas, E. D. Observations on DNA synthesis by bone marrow *in vitro:* stability and pitfalls. In: The Kinetics of Cellular Proliferation, pp. 118–120. Edited by Stohlman, F. New York, 1959. Grune & Stratton.
29. Frenster, J. H., Best, W. R. and Winzler, R. J. Metabolism of human leukocytes *in vitro*. v. Inhibition by human serum of formate and glycine incorporation. *Proc. Soc. Exper. Biol. & Med.*, 98: 887, 1958.
30. Wetherley-Mein, G. and Cottom, D. O. Fresh blood transfusion in leukemia. *Brit. J. Haemat.*, 2: 25, 1956.
31. Bessis, M. and Bernard, J. Remarquables resultats du traitement par l'exsanguino-transfusion d'un cas de leucémie aigue. *Bull. et mém. Soc. méd. Hôp. Paris*, 63: 871, 1947.
32. Bierman, H. R., Kelly, K. H., Centeno, P. A. and Cordes, F. L. The influence of cellular fractions of whole blood on the course of acute lymphocytic leukemia: A preliminary report. In: The Leukemias: Etiology, Pathophysiology and Treatment, pp. 301–316. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press.
33. Weisberger, A. S., Heinle, R. W. and Hannah, R. Transfusion of leukocytes and products of disintegrated leukocytes. *Proc. Soc. Exper. Biol. & Med.*, 70: 749, 1946.

Purine and Pyrimidine Excretion in Normal and Leukemic Subjects*

WILLIAM S. ADAMS, M.D., FRANCES DAVIS *and* MISAE NAKATANI, B.S.
Los Angeles, California

FOR well over a century leukemia has been recognized as a disease entity associated with a marked proliferation of white blood cells. Only in recent years have certain important biochemical differences been demonstrated which typify the leukemic state. Elevated levels of uric acid, an important end product of purine metabolism in man, have been found in the blood serum and urine of many patients with this disease. In many patients with chronic lymphocytic leukemia and in some with acute leukemia the uric acid values for serum and urine are within the range of normal. This finding stimulated these investigations which were designed to determine the urinary purine and pyrimidine products which result from catabolism of nucleoprotein. The comparison between the composition of urine from normal and leukemic subjects is the topic of this report.

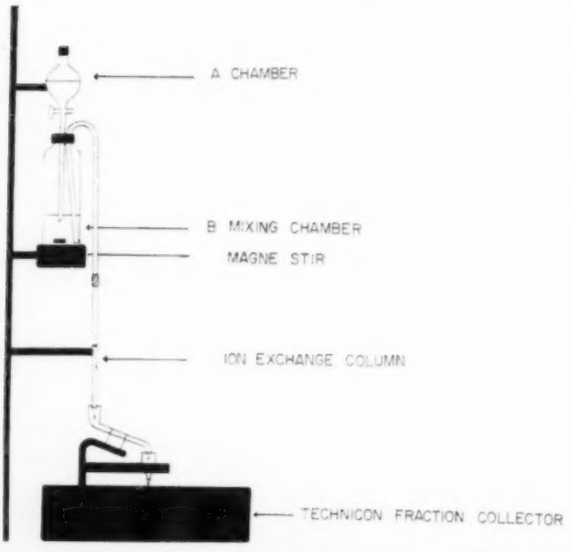


FIG. 1. Diagram of apparatus used for accomplishing gradient elution across Dowex 2 column.

METHODS

In general the methods employed have consisted of a comparison of known authentic purines and pyrimidines with urinary constituents by their elution properties from ion exchange columns, by filter paper partition chromatography and by ultraviolet absorption spectral analysis. The method of column chromatography employed is a modification of the elution technic of Cohn [*1*] and is as follows:

Dowex 2 (anion exchange resin of medium porosity, 8 per cent cross linkage, 200 to 400 mesh, styrene type) is packed to a depth of 24 cm. in a column 0.7 cm. in diameter. The column is washed and charged in the usual manner. One four hundredth of a twenty-four-hour urine collection obtained from a patient kept on a low purine diet for five days is sorbed on the resin bed. Gradient elution is accomplished using an acetic acid, ammonium hydroxide, ammonium chloride system. (Fig. 1.) The mixing chamber B contains a constant volume of 250 cc. which at the start consists of 0.22 molar NH_4OH and 0.25 molar NH_4Cl. By gravity flow a gradual delivery of a solution from separatory funnel A containing 0.1 molar acetic acid and 0.25 molar NH_4Cl results in a gradual fall in pH from 10.4 to 9.5. At the end of approximately 100 fraction cuts of 2.5 cc. each, a solution containing 0.15 molar acetic acid and 0.25 molar NH_4Cl is cut in and an additional 100 fractions collected from the effluent, resulting in a pH decline to 7.0. At the completion of the run the column is stripped with 0.1 N HCl. Scale-up procedures involving larger amounts of urine (100 to 200 cc.) and larger fraction cuts (25 cc.) with appropriate increase in buffer volumes have been accomplished. Recordings of the ultra-violet absorbancy of eluting substances are made at 260 mμ. throughout the entire run and at 280 mμ. as well during the elution of the first peak to circumvent the interference produced by creatine and creatinine.

The methods of filter paper chromatographic separation of concentrated fraction cuts include separation with various solvent systems and filter paper electrophoresis [*2–4*]. Two dimensional separation is conducted using ascending technics, the first phase being

* From the Department of Medicine, School of Medicine, University of California at Los Angeles, and from Wadsworth Hospital, Veterans Administration, Los Angeles, California. This study was supported by the U. S. Public Health Service Grant (CY-2433).

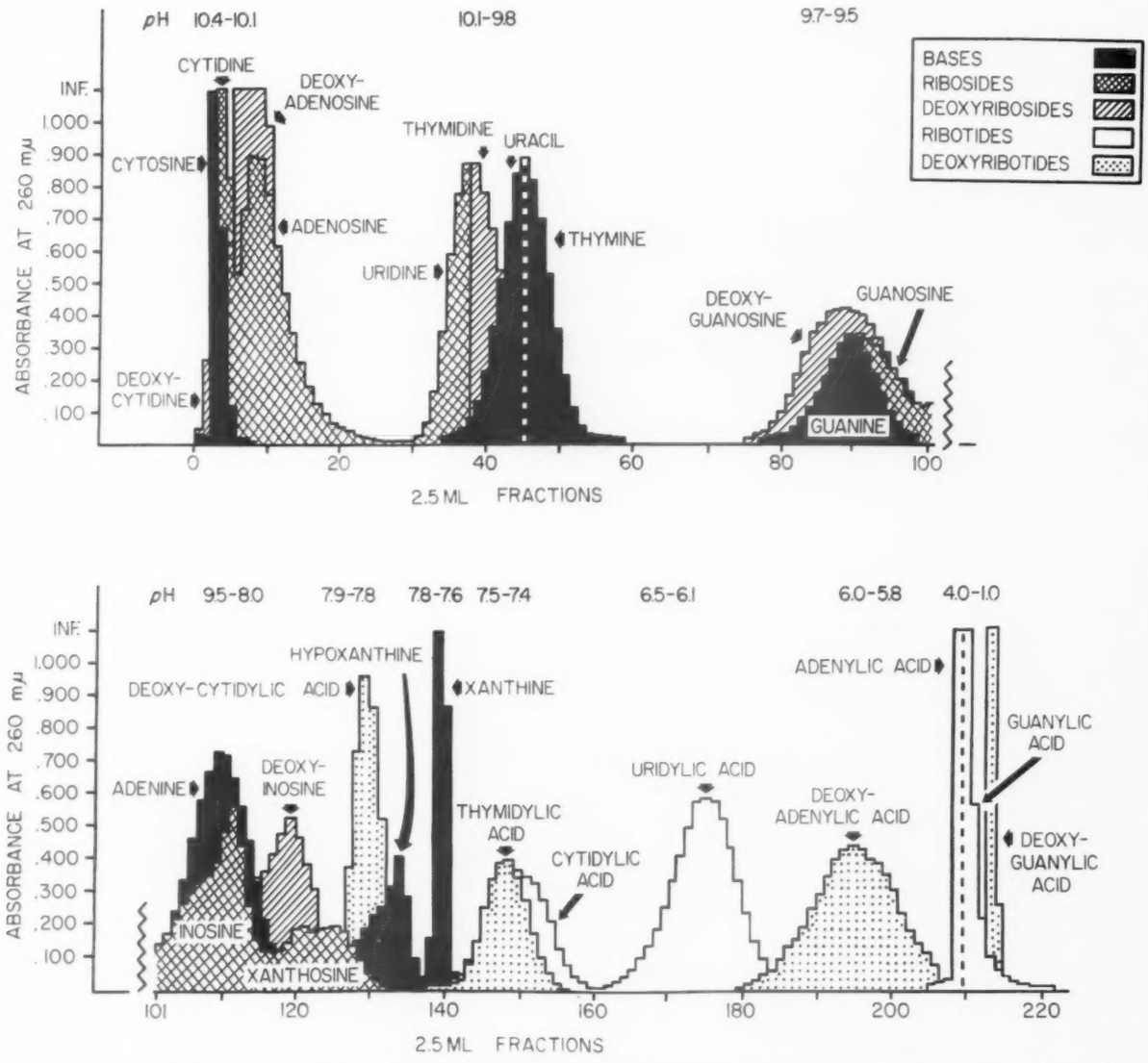


FIG. 2. Sequence of various known standards eluted by pH gradient technic from a Dowex 2 ion exchange column. An average of 0.5 mg. of each standard was applied to the column.

isopropanol and hydrochloric acid, the second phase butanol and ammonium hydroxide. Rf values are computed in the conventional manner.

Absorption spectrum analyses are performed using a recording Beckman DK-2 spectrophotometer. The configuration of the absorption spectrum and the shifts produced by varying the pH plus the determination of isobestic points have been employed in the identification of various unknowns by comparison with authentic standards.

Since many of the elution peaks from urine represent mixtures, it has been necessary to resort to purification procedures involving extracting spots from filter paper and concentrating them by a method modified from Dent [5].

RESULTS

Column Chromatography. The elution sequence of some of the known purine and pyrimidine bases, their ribosides, deoxyribosides, ribotides and deoxyribotides are shown in Figure 2. There is some overlap of the area of elution of some of these compounds, and it has been found that the exact position of elution may vary depending upon the amount and number of substances sorbed on the column, slight pH differences and temperature. Hence the results of column chromatographic separation can be accepted only as a gross means of identification of an unknown.

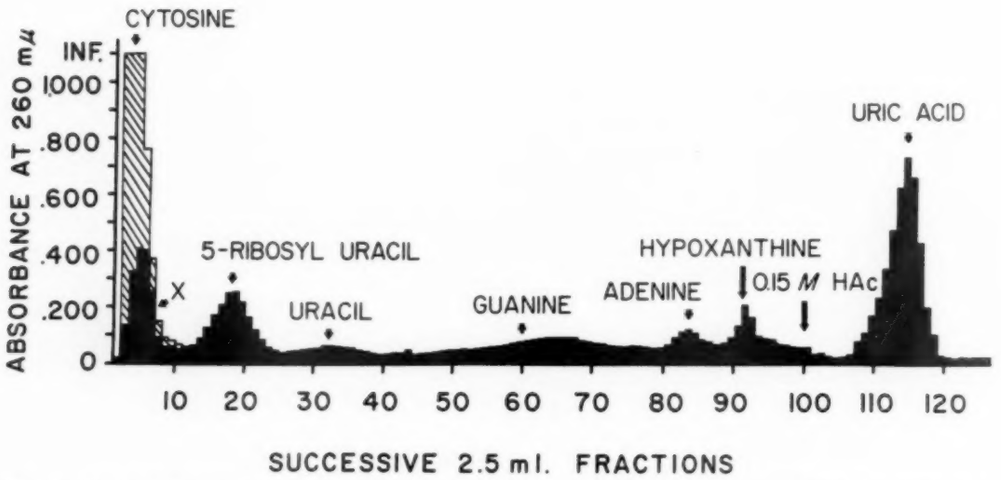


FIG. 3. Elution diagram of various purine and pyrimidine compounds obtained from the urine of a normal subject placed on a low purine diet for five days. Absorbance was measured at 260 mμ. with the exception of the solid black area of the first peak on the left which was measured at 280 mμ. because of the interference produced by creatinine. This is shown as a cross hatched area.

The results of column chromatography of urine from normal subjects and from those with leukemia are shown in Figures 3 through 8. To date urine from ten normal subjects and forty patients with different types of leukemia have been analyzed. However, for the sake of brevity, and because there has been considerable comparability of results, only the data on a normal male subject and four patients with leukemia are shown. It will be noted in the figures that not all peaks are labeled since sufficient information has not been obtained to identify all eluting substances. Furthermore, no data are reported for the ribotide area of elution after 0.1 N HCl is passed through the column since time has not permitted a thorough study of this area.

The results can best be described by referring to the various peaks.

Peak 1: This peak represents a mixture of trace amounts of cytosine and large amounts of creatinine (cross hatched area) which contribute to the regular occurrence of readings in the infinity when read at 260 mμ. but which is circumvented by reading at 280 mμ. In addition, this peak contains an unknown substance designated as X. The absorption spectrum of this unknown is shown in Figure 9 which depicts the minimal shifts resulting from varying the pH.

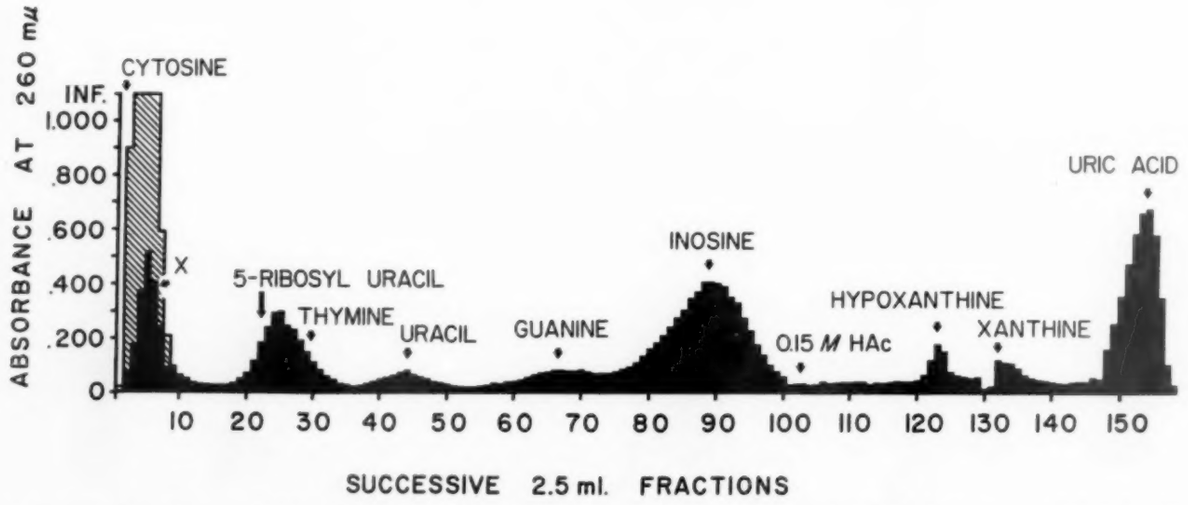


FIG. 4. Elution diagram obtained from a patient with chronic lymphocytic leukemia. Note the presence of thymine in the descending limb of the second peak from the left and the presence of large amounts of inosine.

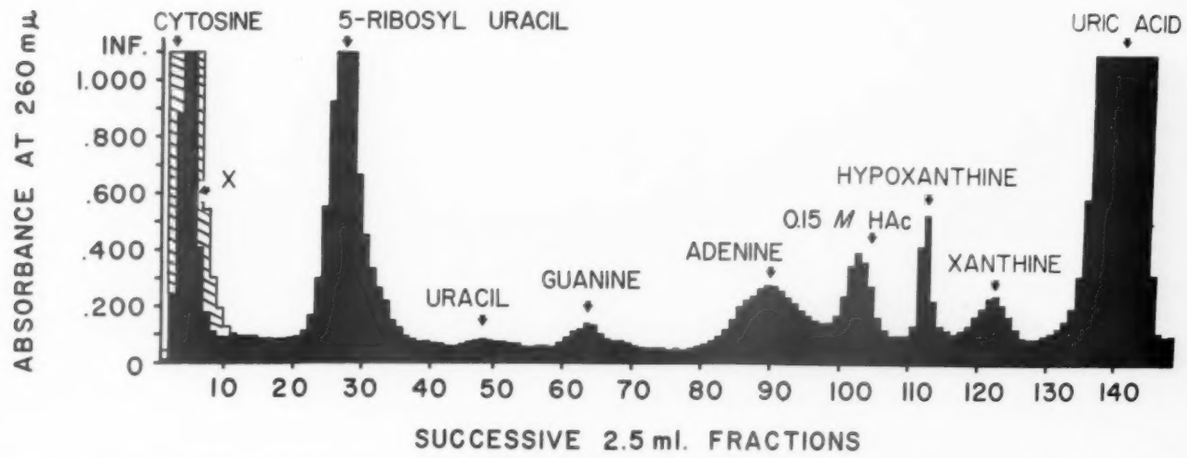


FIG. 5. Elution diagram obtained from a patient with chronic myelocytic leukemia in blastic relapse. Note the large amounts of X, 5-ribosyl-uracil and increased purine excretion.

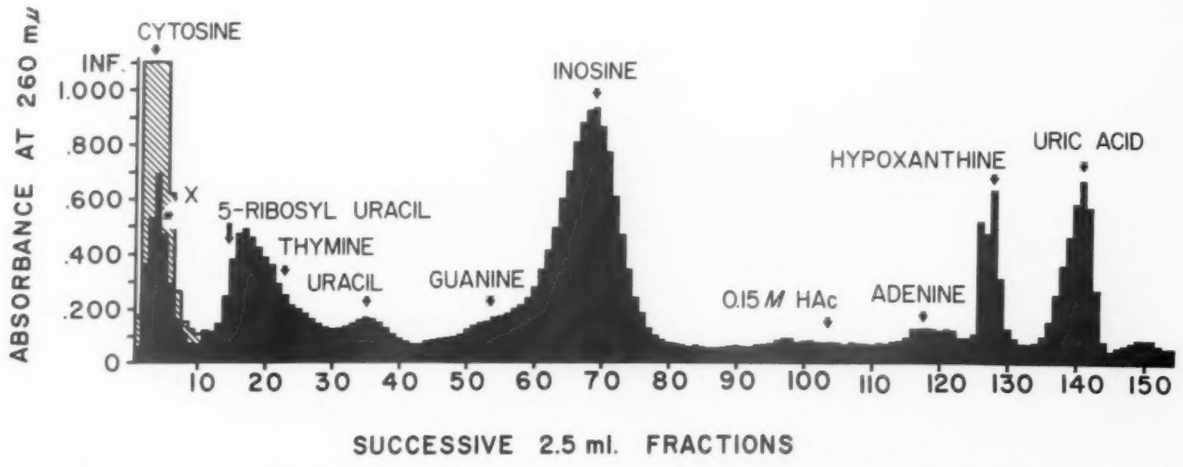


FIG. 6. Elution diagram obtained from a patient with acute leukemia, probably myelocytic in type. Note particularly the increased excretion of 5-ribosyl-uracil and inosine. Thymine was present in the descending limb of the second peak from the left. Note also the relatively low excretion of uric acid.

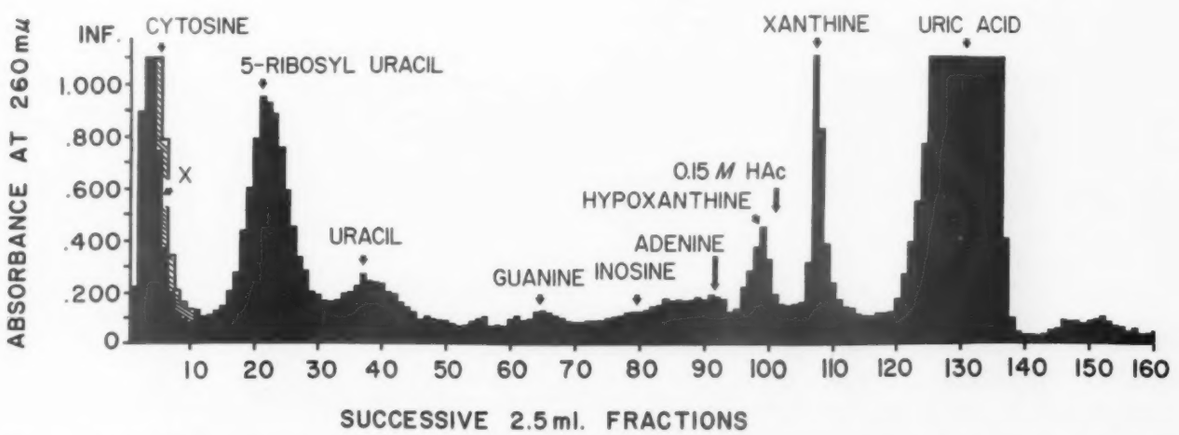


FIG. 7. Elution diagram obtained from a patient with acute leukemia, probably monocytic in type, during a phase of relapse. Note increased excretion of X, 5-ribosyl-uracil, uracil and uric acid.

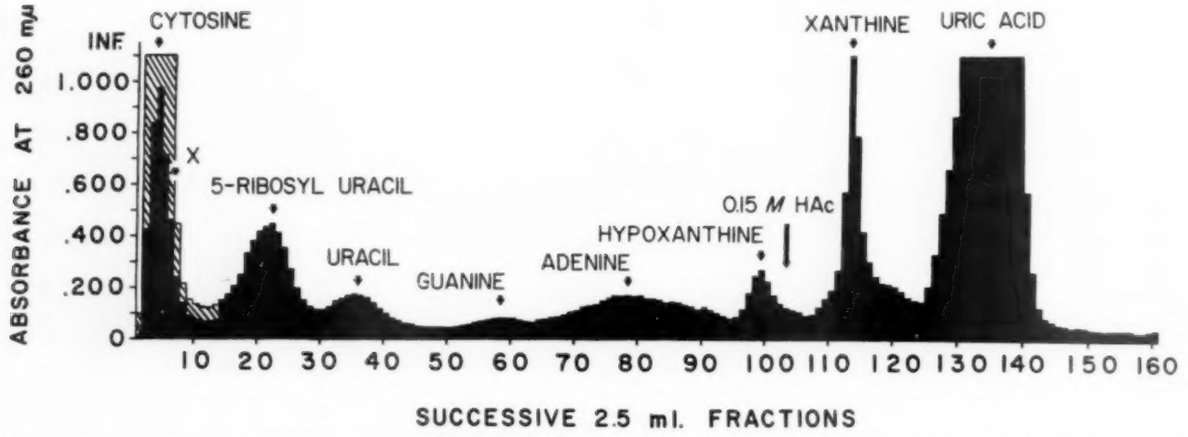


FIG. 8. Elution diagram from the same patient as shown in Figure 7 during a period of partial remission induced by the administration of 6-mercaptopurine. Note the decrease in amounts of X and 5-ribosyl-uracil.

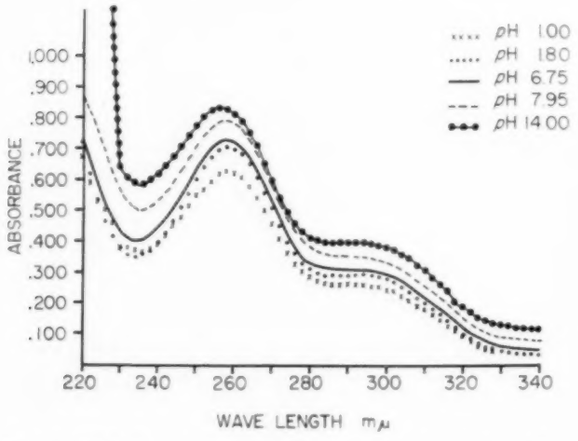


FIG. 9. This figure shows the absorption spectrums of a substance designated as X isolated from urine and eluting in the first ten fractions from a Dowex 2 column. Note the minimal shifts produced by varying the pH. Suggested possible formula is N-methyl-2-pyridone-5-carboxamide.

It can be seen that the maximal absorption is at 258 mμ with a strong shoulder at 280 to 300 mμ.

Rf values determined from filter paper chromatography are as follows:

Isopropanol and hydrochloric acid	0.85
N-butanol and ammonia	0.46
Isoamyl alcohol and sodium phosphate	0.00
N-butanol and formic acid	0.06
N-butanol and boric acid	0.00 0.52

On hydrolysis with 6 N HCl for twenty-four hours at 120°C., this compound is only partially hydrolyzed as revealed by filter paper chromatography. However, three ninhydrin spots appear on two dimensional filter paper chromatography in tertiary butanol, formic acid and phenol solvent systems and have the same Rf values as glycine, glutamic acid and alpha-alanine.

This compound has been found to be increased approximately twofold in chronic granulocytic leukemia in blastic relapse and in acute myelocytic leukemia. (Table 1.)

Peak II: This peak consists mainly of 5-ribosyl-uracil (pseudouridine). In a few cases (acute and chronic lymphocytic leukemia) thymine is found to elute in the descending limb of this peak. An absorption spectrum analysis for 5-ribosyl-uracil obtained from urine is shown in Figure 10. Rf values for this urinary constituent compared to standard 5-ribosyl-uracil* are as follows:

	5-ribosyl-uracil	Urinary Constituent
86% N-butanol, H_2O	0.47	0.47
Tertiary amyl alcohol, formic acid	0.68	0.66
Isopropanol, acetic acid	0.85	0.85

When the urinary constituent and standard 5-ribosyl-uracil were spotted at the origin of a two dimensional paper chromatogram (isopropanol, hydrochloric acid; n-butanol and ammonia), both travel as a single spot. Normal subjects excreted approximately 60 mg. of this substance in twenty-four hours, whereas all patients with leukemia thus far studied have shown an increased excretion. (Table 1.) In one patient with chronic myelocytic leukemia in

* Supplied through the courtesy of Dr. Waldo Cohn.

blastic relapse as much as 416 mg. was found in a twenty-four-hour urine specimen. (Fig. 5.)

Peak III: This peak consists of uracil alone. The absorption spectrums and Rf values in various solvent systems are identical with known standards. Quantitative excretion data indicate that in most patients with leukemia excretion of uracil is increased. In one patient with acute monocytic leukemia as much as 75 mg. were excreted in a twenty-four-hour period. (Fig. 7 and Table I.)

Peak IV: This area of elution contains guanine and shows little quantitative fluctuation from normal values in the leukemic state. (Table I.)

Peak V: Inosine, the riboside of hypoxanthine, was recovered in large amounts from the urine of a patient with acute myelocytic leukemia (Fig. 6) and of a patient with chronic lymphocytic leukemia. (Fig. 4.) Using the technic described in this report, it has not been recovered from the urine of normal subjects.

Peaks VI through VIII: These areas of elution represent mixtures and the data recorded in the figures and table must be considered as approximations. The figures have been labeled with the major absorbing compound reported. Adenine, hypoxanthine and xanthine have been identified in the urine as shown. In one patient with chronic myelocytic leukemia in blastic relapse a substance which has the spectral characteristics and Rf values of guanosine was found and eluted in fractions 100 through 105 but it is not labeled because of an admixture with other purine compounds. (Fig. 5.)

Peak IX: This peak contains uric acid. Its excretion is increased in two of the patients described, one with acute monocytic leukemia and the other with chronic myelocytic leukemia in blastic relapse. However, in two other patients, one with chronic lymphocytic leukemia and the other with acute myelocytic leukemia, uric acid excretion was not increased. Uric acid was identified as the major constituent of this peak by its characteristic absorption spectrum, its Rf values and by its reaction with uricase.

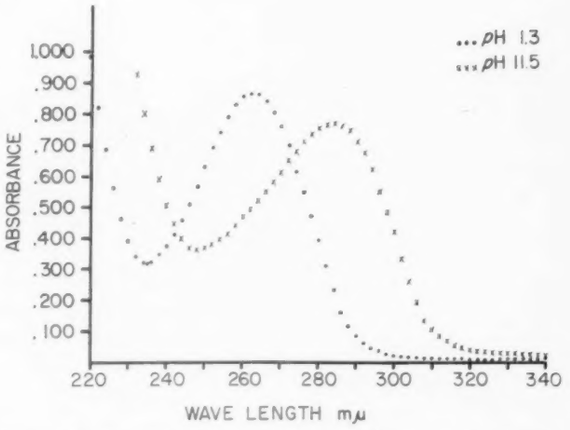


FIG. 10. Absorption spectrums obtained for 5-ribosyl-uracil isolated from urine.

COMMENTS

Studies of purine excretion, other than uric acid, date from 1898 when Krüger and Salomon [6,7] isolated hypoxanthine, xanthine, adenine and 7-methyl-guanine from 10,000 L. of human urine. However, progress in this field of investigation was limited by the lack of appropriate

TABLE I*

QUANTITATIVE URINARY DETERMINATIONS IN NORMAL AND LEUKEMIC SUBJECTS

Compound	Normal Male	Acute Leukemia		Acute Leukemia (P. K.)	Chronic Lymphocytic Leukemia (S. G.)	Chronic Myelocytic Leukemia-Blastic Relapse (H. H.)
		Relapse (A. M.)	Partial Remission Induced by 6-MP (A. M.)			
Compound X	45	85	103	63	49	330
5-ribosyl-uracil	62	358	170	204	91	416
Uracil	7	75	43	32	11	13
Guanine	22	28	15	...	19	25
Inosine	...	.....	.....	316	153	.....
Adenine	4	60	30	10	...	39
Hypoxanthine	8	32	19	35	10	24
Xanthine	...	80	60	...	14	24
Uric acid	390	1,851	1,452	347	443	1,903

* All values are given in mg./twenty-four hours.

methods of isolation, separation and identification; it was not until 1947 that Vischer and Chargaff [*8*] and Hotchkiss [*9*] reported the feasibility of separating nucleic acid derivatives using filter paper chromatography. Cohn [*1*] adapted the method of ion exchange chromatography to the same problem thus making it possible to separate, identify and quantitate these compounds from several biologic systems. In 1954 Weissmann et al. [*10*] reported preliminary findings on the chromatographic investigation of purines in human urine. Subsequent reports from the same investigators [*11,12*] substantiated the presence of hypoxanthine, xanthine, adenine and 7-methyl-guanine and for the first time mentioned the presence of 1-methyl-guanine, N-2 methyl-guanine, 1 methyl-hypoxanthine and 8-hydroxy-7-methyl-guanine.

Information concerning pyrimidine excretion in man has been more fragmentary. In 1954 Horrigan [*13*] reported the increased excretion of uracil in the urine of patients with chronic myelocytic leukemia. Recently a new pyrimidine, 5-ribosyl-uracil, has been identified [*14*] and reported in human urine by Adler and Gutman [*15*].

The data presented in this report confirm some of the earlier investigations. However, because of differences in methodology and because of a different focus of interest, namely, that of attempting to detect major differences in the excretory pattern as related to disease, these findings vary from previously reported information.

The as yet unidentified ultraviolet absorbing compound designated as X in the figures and table deserves some comment. It is of interest because its increased excretion in some patients with leukemia warrants careful study. Recently it has been suggested that this substance is N-methyl-2-pyridone-5-carboxamide. Should it prove to be this compound, a metabolite of nicotinamide and tryptophane metabolism, it would not afford as much interest as it would if it were to be a substituted pyrimidine or purine. Final identification must await further study.

The major constituent of the second eluting peak has proved to be 5-ribosyl-uracil. For some time it was apparent that, contrary to what had been reported previously, the major uracil derivative present in this peak was dissimilar from standard uracil in three respects. (1) It eluted from the column in fractions 15 through 30 rather than in fractions 40 through 50. (2) The absorption spectrums for 5-ribosyl-uracil (Fig. 10) at pH 1.3 shows a maximum of 262 mμ., a minimum of 235 mμ. and at pH 11.5, 286 mμ., respectively. These figures differ from the spectrums obtained for standard uracil in that the shift is slightly to the right. (3) The Rf values for 5-ribosyl-uracil in various solvent systems differed from uracil standards. For the same reasons it was determined that this eluting fraction was not standard uridine. It was not until Davis and Allen [*14*] reported the discovery of a new uridine isomer obtained from an extract of yeast nucleic acid that the major urinary component reported here was thought to be 5-ribosyl-uracil. This proved to be the case and when compared to a standard provided by Dr. Waldo Cohn the two are found to be identical. It is noteworthy that the excretion of 5-ribosyl-uracil is invariably increased in all patients with leukemia thus far studied. In one patient with chronic myelocytic leukemia in blastic relapse as much as 416 mg. were excreted in twenty-four hours; this is to be compared to a normal value of 60 mg. In most leukemic subjects, however, the range of excretion is intermediate varying from 100 to 300 mg. per day. When a normal subject is fed a normal diet containing beef protein, the excretion of 5-ribosyl-uracil increases. When the same subject is fed a low purine diet in which the major source of dietary protein comes from dairy products, the excretion of 5-ribosyl-uracil levels off at 50 to 70 mg. per day. The consistently increased excretion of 5-ribosyl-uracil in leukemic subjects fed low purine diets invites further study and explanation.

As noted previously thymine has been recovered in small amounts from the urine of patients with chronic lymphocytic leukemia and from some patients with acute leukemia. In one patient with acute lymphocytic leukemia, in whom a complete hematologic and clinical remission was obtained with 6-mercaptopurine, thymine was found in the urine. However, when the same subject was studied during a period of relapse, thymine no longer was present. This was the only difference which was detected under the circumstances of complete remission and relapse. It is to be admitted that single cases may mean relatively little. However, the opportunity of studying complete remission and relapse in the adult is not encountered frequently.

The increased excretion of uracil in patients with chronic myelocytic leukemia has been reported previously [*13*]. This study confirms

these observations and shows that this abnormality is also present in patients with other types of leukemia. (Table 1.) Small amounts of uracil are found in the urine from normal subjects (6 to 8 mg. per day). Two- to tenfold excretion of uracil may be encountered in urine from patients in the leukemic state. It is to be emphasized however, that the major uracil-containing fraction is 5-ribosyl-uracil, not uracil or uridine. Under the circumstances of these studies no detectable amounts of uridine were recovered. It is presumed that the recovery of uracil in increased amounts from the urine of leukemic subjects reflects an augmented production of this pyrimidine which is not completely catabolized and hence spills in the urine in increased amounts.

The isolation, separation and identification of the purine bases, guanine, adenine, hypoxanthine and xanthine from urine have presented certain difficulties because of mixing of the various fraction cuts eluted from the column. The data as presented in the figures are given for the major ultra-violet absorbing purine bases present in the various peaks. It is recognized that the data given in Table 1 are only semiquantitative because of the problem mentioned herein. It appears that the excretion of guanine in patients with leukemia is not markedly altered from that in normal subjects, whereas excretion of adenine, hypoxanthine and xanthine frequently are increased. Of considerable interest is the marked increase in the excretion of inosine, the riboside of hypoxanthine, in two situations, i.e., chronic lymphocytic leukemia and acute myelocytic leukemia. Since the excretion of uric acid, hypoxanthine and xanthine was not markedly increased in these two patients it would appear that the action of the enzyme, inosine nucleosidase, is in some manner defective.

Increased excretion of uric acid has long been known to be associated with some cases of leukemia [*16*]. However data accumulated in this laboratory [*17*] and elsewhere [*18*] have indicated that this is not an invariable accompaniment of this disease. Uric acid excretion was not increased in either the patient with chronic lymphocytic leukemia or in the patient with acute myelocytic leukemia. In contradistinction to these two cases is the marked excretion of uric acid in the patient with chronic myelocytic leukemia in blastic relapse (1,903 mg. per day) and in the patient with acute monocytic leukemia (1,851 mg. per day). It is frequently difficult to interpret observations made of the quantitative excretion of uric acid since dietary fluctuations influence such findings and since uric acid can be formed from simple precursors (glycine, formate and CO_2) which are not involved with the catabolism of nucleic acids. It is probable that future studies of uric acid excretion may have more validity as a measure of cell breakdown or cell turnover if they are combined with quantitation of other purine precursors and the pyrimidine 5-ribosyl-uracil.

SUMMARY

The excretion of various purine and pyrimidine compounds in the urine of one normal and four leukemic subjects is reported. Additional data obtained from ten normal subjects and forty patients with leukemia have not been reported since much of the information accumulated is duplicated. The methods which have been utilized are described in some detail. Possibly such methods may be applicable to other biologic systems, such as blood serum and cell extracts.

Acknowledgment: We gratefully acknowledge the advice and assistance of Drs. George Brown, Waldo Cohn, Clyde Dubbs, Robert Fink, Kay Fink and William Skoog.

REFERENCES

1. Cohn, W. E. The separation of purine and pyrimidine bases and nucleotides by ion exchange. *Science*, 109: 377, 1949.
2. Wyatt, G. R. The purine and pyrimidine composition of deoxypentose nucleic acids. *Biochem. J.*, 48: 584, 1951.
3. MacNutt, W. S. The enzymically catalyzed transfer of the deoxyribosyl group from one purine or pyrimidine to another. *Biochem. J.*, 50: 384, 1952.
4. Semenza, G. Separation of purines and pyrimidines by paper electrophoresis. *Acta chem. scandinav.*, 11: 24, 1957.
5. Dent, C. E. The aminoaciduria in the Fanconi syndrome. *Biochem. J.*, 41: 240, 1947.
6. Krüger, M. and Salomon, G. Die Alloxurbasen des Harnes. *Ztschr. f. physiol. Chem.*, 24: 364, 1898.
7. Krüger, M. and Salomon, G. Die Alloxurbasen des Harnes. *Ztschr. f. physiol. Chem.*, 26: 350, 1898.
8. Vischer, E. and Chargaff, E. The separation and characterization of purines in minute amounts of nucleic acid hydrolysates. *J. Biol. Chem.*, 168: 781, 1947.
9. Hotchkiss, R. D. The quantitative separation of purines, pyrimidines and nucleosides by paper chromatography. *J. Biol. Chem.*, 175: 315, 1948.
10. Weissmann, B., Bromberg, P. A. and Gutman, A. B. Chromatographic investigation of purines in nor-

mal human urine. *Proc. Soc. Exp. Biol. & Med.*, 87: 257, 1954.

11. Weissmann, B., Bromberg, P. A. and Gutman, A. B. The purine bases of human urine. I. Separation and identification. *J. Biol. Chem.*, 224: 407, 1957.
12. Weissmann, B., Bromberg, P. A. and Gutman, A. B. The purine bases of human urine. II. Semi-quantitative estimation and isotope incorporation. *J. Biol. Chem.*, 224: 423, 1957.
13. Horrigan, D. L. Urinary excretion of nucleic acid derivatives in persons with myeloid leukemia. *J. Clin. Invest.*, 33: 901, 1954.
14. Davis, F. F. and Allen, F. W. Ribonucleic acids from yeast which contain a fifth nucleotide. *J. Biol. Chem.*, 227: 907, 1957.
15. Adler, M. and Gutman, A. B. Uridine isomer (5-ribosyl-uracil) in human urine. *Science*, 130: 862, 1959.
16. Salkowski, E. Beiträge zur Kenntniss der Leukämie. *Virchows Arch.*, 50: 174, 1870.
17. Adams, W. S., Figueroa, W. G., Skoog, W. A. and Lawrence, J. S. Uric acid excretion in various hematologic disorders. Unpublished data.
18. Sandberg, A. A., Cartwright, G. E. and Wintrobe, M. M. Studies on leukemia. I. Uric acid excretion. *Blood, J. of Hematology*, 11: 154, 1956.

Mechanisms of Drug Action in Leukemia*

IRWIN H. KRAKOFF, M.D.
New York, New York

THE mechanisms by which various materials influence cell growth have been studied in a large and diverse group of biologic systems, e.g., cellular or cell-free tissue homogenates, bacteria, mammalian cells in tissue culture, and transplanted leukemias and tumors in mice and rats. These systems have yielded information as to the extent and mechanism of inhibition of cell growth by various compounds. This information, however, cannot invariably be extrapolated to leukemia in man and, even when this is possible, not necessarily to all types of leukemia in man since the metabolic processes of the cells involved may be dissimilar. Nevertheless, agents found to be effective in inhibiting growth in biologic systems can produce clinical benefit in some types of leukemia and the mechanisms of these effects appear, in some cases, similar to the mechanisms observed in the simpler systems.

Since nucleic acids play an important part in the regulation of cell growth, attempts to develop agents capable of modifying cell growth have focused largely on substances which might be capable of destroying or inactivating nucleic acids or inhibiting their biosynthesis. To develop such materials, compounds have been synthesized which are structurally related to the various moieties of the nucleic acids in order to block the synthesis or utilization of these essential components. Some of these analogs have become clinically useful although, in general, clarification of their precise modes of action has followed rather than preceded the demonstration of their clinical usefulness. "Empirical" screening programs have been developed to test the activity of thousands of such chemical and biologic substances for antineoplastic activity. Of those found to possess such activity, some have subsequently been shown to act by inhibiting one or another aspect of nucleic acid biosynthesis.

The nucleic acids are large aggregates of purine and pyrimidine ribonucleotides, with molecular weights varying from several hundred thousand to several million. Ribonucleic acid (RNA) occurs principally in the cytoplasm and nucleoli of cells. Deoxyribonucleic acid (DNA) is found only in the nucleus and is thought to be the material in the chromosomes which transmits genetic information. The two substances differ in composition and structure [*1*]. RNA has been thought to be composed of either a long, branching chain of purine and pyrimidine nucleotides [*2*] or of mixtures of shorter chains [*3*]. There are significant differences in structure between the soluble RNA of cytoplasm and the particulate RNAs of nucleoli and microsomes. DNA is currently thought to consist of twin, linked chains of purine and pyrimidine nucleotides arranged in a double helical pattern [*4*]. The differences in the sugars and pyrimidine bases of RNA and DNA are shown in Table 1.

There is constant replacement and renewal of the constituents of the nucleic acids, either by turnover within the living cell (in the case of RNA) or by aging and breakdown of the cell with degradation of the nucleic acids and con-

TABLE 1
PRINCIPAL CONSTITUENTS OF THE NUCLEIC ACIDS

Ribonucleic Acid	Deoxyribonucleic Acid
Adenine	Adenine
Guanine	Guanine
Cytosine	Cytosine
Uracil	Thymine
Ribose	2′-Deoxyribose
Phosphate	Phosphate

* From the Division of Clinical Chemotherapy, Sloan-Kettering Institute for Cancer Research; Department of Medicine, Memorial and James Ewing Hospitals, and Cornell University Medical College, New York, New York.
This work was supported by Research Grant CY3215 from the National Cancer Institute, U. S. Public Health Service; by a grant from the American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research; and institutional grants from the Lasker Foundation and Grant Foundation.

stant biosynthesis of purines and pyrimidines to form new molecules of nucleic acids for the formation of new cells. The rates of synthesis and degradation of DNA are related to the rates of growth, aging and death of cells and are therefore rapid in rapidly growing normal and neoplastic tissues and slow in the tissues which are slow in growth.

Although it has been suggested that there may be differences between the nucleic acids of normal and neoplastic cells, consistent differences, which could be specifically exploited by chemotherapeutic agents, have not been described. With perhaps the single exception of the adrenal cortical hormones in acute leukemia, no compounds have been tested which have been shown to act specifically on leukemic cells, without affecting normal hematopoiesis. Whatever differences in response may exist are quantitative and minor, requiring careful titration of dosage in order to achieve maximum effects on neoplastic cells without causing serious and irreversible damage to normal cells.

The chemical substances which have been shown to have some value in the treatment of clinical or experimental leukemia are classified in Table II.

TABLE II
CLASSIFICATION OF ANTILEUKEMIC COMPOUNDS

I.	Antimetabolites
	Folic acid antagonists
	Glutamine antagonists
	Purine analogs
	Pyrimidine analogs
	Urethan (?)
II.	Polyfunctional alkylating agents
III.	Adrenal cortical steroids
IV.	Miscellaneous compounds
	Colchicine analogs
	Potassium arsenite
	Other hematopoietic depressants

Antimetabolites. Antimetabolites are structural analogs of physiologically occurring substances (metabolites) which can produce evidence of deficiency of the metabolites in a biologic system [*5*]. This does not imply that all structural analogs are antimetabolites, since many are metabolically inert and some may even substitute for the corresponding metabolite. This definition also requires that the analog interfere with the function of the corresponding physiologic substance. Dozens of such substances are known and some are employed in various aspects of medical practice. Among these are vitamin K and Dicumarol,® and histamine and Benadryl®; examples of antimetabolites used to modify, for therapeutic purposes, the activity of a physiological compound [*5*]. An example more closely related to leukemia chemotherapy is the relationship between paraaminobenzoic acid (PABA) and its closely related structural analog, sulfanilamide [*5*]. Certain bacteria require PABA for folic acid synthesis, and if sulfanilamide is supplied PABA is not utilized, thus inhibiting growth. In this instance, however, there is a qualitative difference between the invading bacterial cell and the host, which can be exploited to eradicate the bacteria, since man does not synthesize folic acid but receives it from exogenous sources and therefore does not require PABA. The administration of sulfanilamide therefore produces no toxicity, except for occasional idiosyncrasies and problems resulting from crystallization in the urine. As already indicated, no such qualitative differences have been found between leukemic cells and normal host tissues. There are, at best, relatively minor quantitative differences which are not necessarily related to neoplasia but rather to the growth rates and metabolic characteristics of the involved cells.

Figure 1 gives examples of proved or potentially useful antimetabolites, compared structurally with the corresponding metabolite.

Folic Acid Antagonists. Since the discovery, in 1948 [*6*], that aminopterin, an analog of folic acid, is capable of inducing remissions in children with acute leukemia, there has been intensive investigation of the mechanism of action of this class of compounds. It was shown in leukemic animals that the folic acid antagonists could markedly inhibit the incorporation of isotopically labeled precursors into the nucleic acid purines of normal and leukemic tissues [*7*]. The metabolic site of this inhibition has been shown to be in the *de novo* synthesis of purines, specifically in preventing the formylation of GAR to form FGAR and the formylation of AICAR to form inosinic acid (Fig. 2), the first complete purine formed in the synthetic pathway and the intermediate from which other purines are thought to be derived. The folic acid antagonists block these steps by inhibiting the transfer of single-carbon (formyl) groups into the 8 and 2 positions of the purine ring [*8–13*]. This transfer is accomplished by a complex series of reactions which are summarized

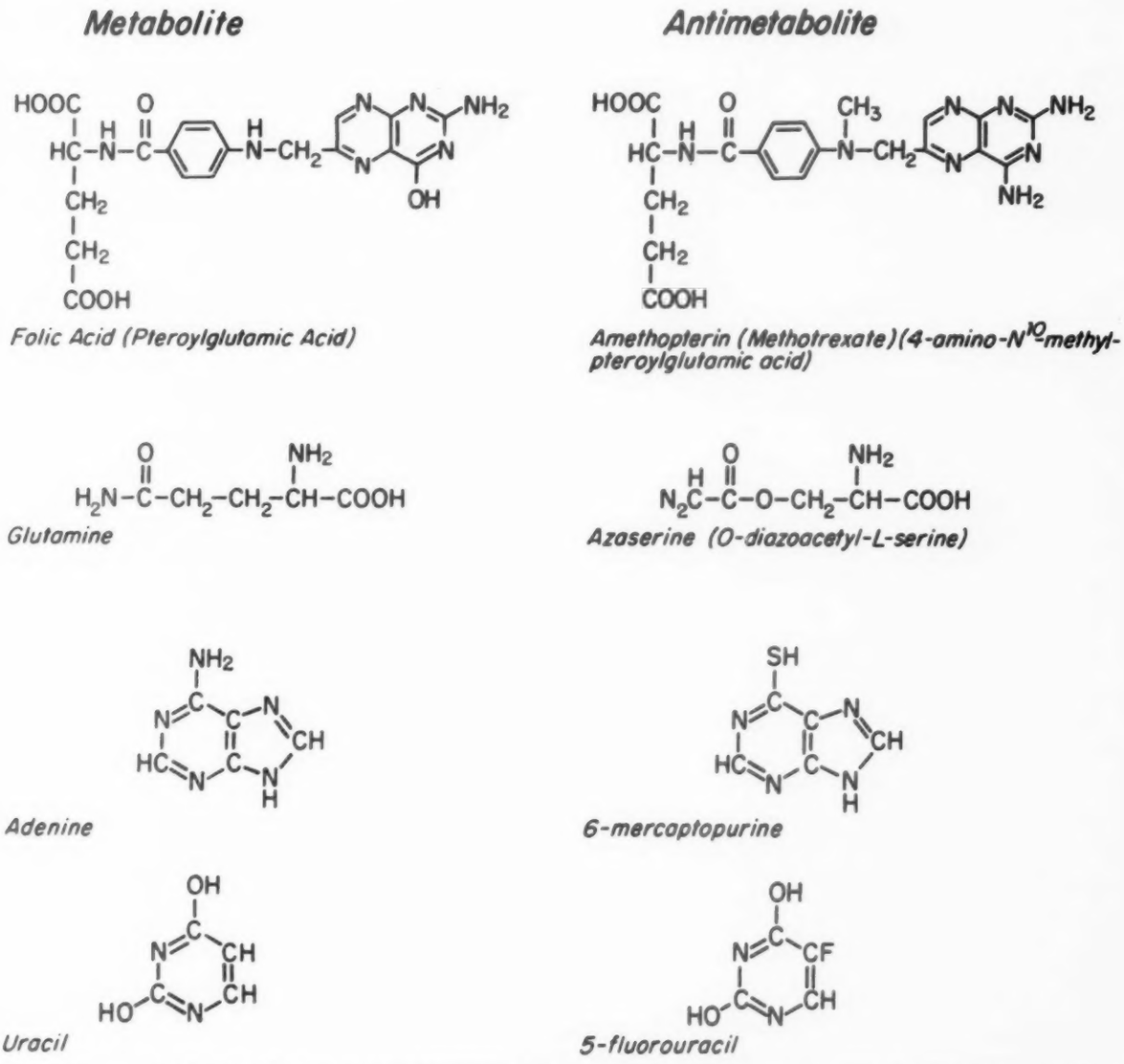


FIG. 1. Structural formulas of some of the substances involved in nucleic acid synthesis, and of some antimetabolites known to function as inhibitors of nucleic acid synthesis in various systems.

in Figure 3. In this reaction, citrovorum factor (CF) may be identical with N^{10}-formyl THFA or may be a precursor. Different "folic acid antagonists" may act at different sites in this series of reactions. Aminopterin (4-amino folic acid) is thought to block the reduction of FA to THFA, whereas amethopterin (4-amino-N^{10}-methyl folic acid) appears to block the conversion of THFA to N^{10}-formyl THFA [*12*].

The antileukemic effect and toxicity of these compounds in both experimental animals and man can be prevented by the prior administration of small doses of CF or large doses of folic acid [*14–16*]. It has not been possible, however, to reverse the toxicity of the antifolics once it has developed or to prevent their toxic effects by prior administration of folic acid or CF without concomitant prevention of the antileukemic effect.* It has been demonstrated in several biologic systems that the effects of these com-

* A recent report [*17*] has indicated that local, intra-arterial administration of large doses of amethopterin, together with systemic administration of citrovorum factor, can cause local regression of tumors (all carcinomas in this series) with minimal systemic toxicity. This is attributed to a gradient in concentrations of the antimetabolite and metabolite due to the different routes of administration rather than a metabolic separation of effects.

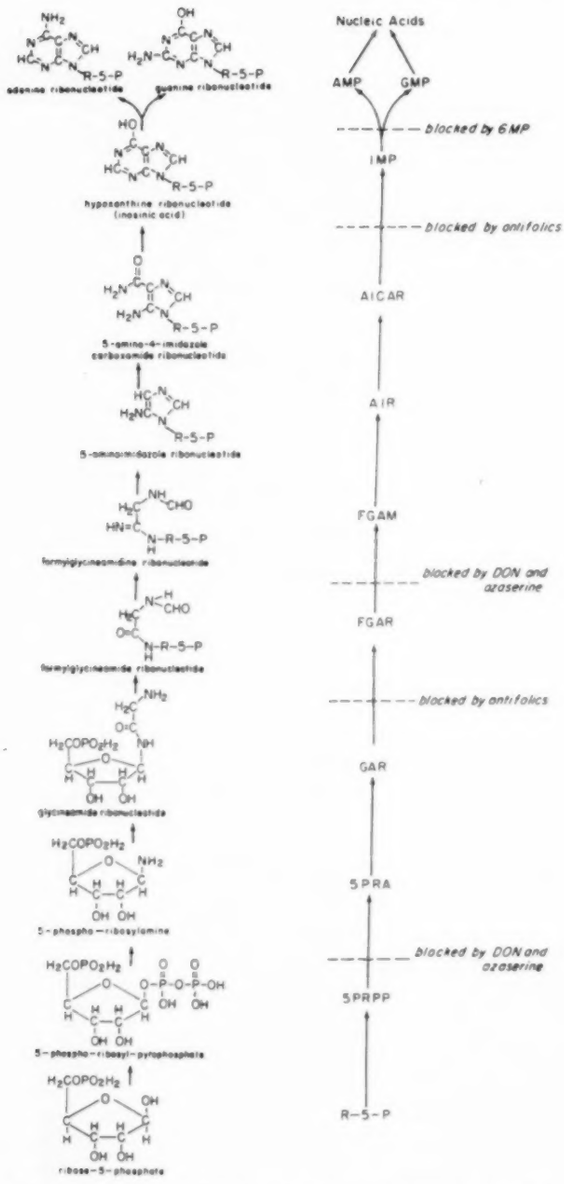


FIG. 2. Outline of the pathway of purine biosynthesis. On the left are the structural formulas of the compounds involved. On the right are the usual abbreviations (these are not always consistent but conform with common usage) and indications of the sites of action of antimetabolites affecting this system.

pounds can be prevented by the administration of adenine. This indicates that the results of inhibiting *de novo* synthesis of purines are alleviated by supplying an exogenous purine.

An additional site of action of these compounds is in pyrimidine biosynthesis. (Fig. 4.) Here a folic acid-derived compound is necessary for a carbon transfer in converting uridylic acid to the 5-methyl derivative, thymidylic acid [*18*].

(1) Folic Acid $\xrightarrow{+2H}$ Dihydrofolic Acid (DHFA)

(2) DHFA $\xrightarrow{DPNH}$ Tetrahydrofolic Acid (THFA)

(3) THFA $\xrightarrow{formate}$ N^{10}-formyl THFA

(4) N^{10}-formyl THFA + GAR $\longrightarrow$ FGAR + THFA

or

(4) N^{10}-formyl THFA + AICAR $\longrightarrow$ IMP + THFA

FIG. 3. Outline of the reactions by which folic acid and its derivatives participate in transfer of single carbon units. The step shown here as (3) is itself a complex one involving unstable intermediates [*13*].

In the developing chick embryo the antifolic effects can be partially prevented by large doses of thymidine [*19*], even though this site appears to be quantitatively less important than the effects on purine biosynthesis.

The antifolics have also been shown to block the incorporation of single carbon fragments into various amino acids and coenzymes, transfers which are presumably also dependent on a folic acid-derived substance. It has not been determined if these latter functions have any significance in relation to the antileukemic effects.

In man resistance has eventually developed in all cases of leukemia which responded initially to therapy with the folic acid antagonists resulting in failure of continued control of the disease. This resistance is analogous to that which sometimes appears in bacteria after exposure to antibiotics. In transplanted mouse leukemias it has been possible to develop leukemic cell lines which are resistant to antifolic therapy by exposing multiple successive transplant generations to subcurative doses [*20,21*]. This has been considered to be due to the emergence of mutant strains of cells with metabolic pathways different from those of the parent strain. It has been shown that amethopterin-resistant bacteria can convert folic acid to CF much more readily than the parent, sensitive strain [*22*]. The resistant organism also appears to have developed a greater requirement for preformed purines than the sensitive one [*23*].

Resistance to one folic acid antagonist appears to produce resistance to all 4-amino analogs but not to other classes of antimetabolites in either experimental systems or in man since sequential remissions in children with leukemia

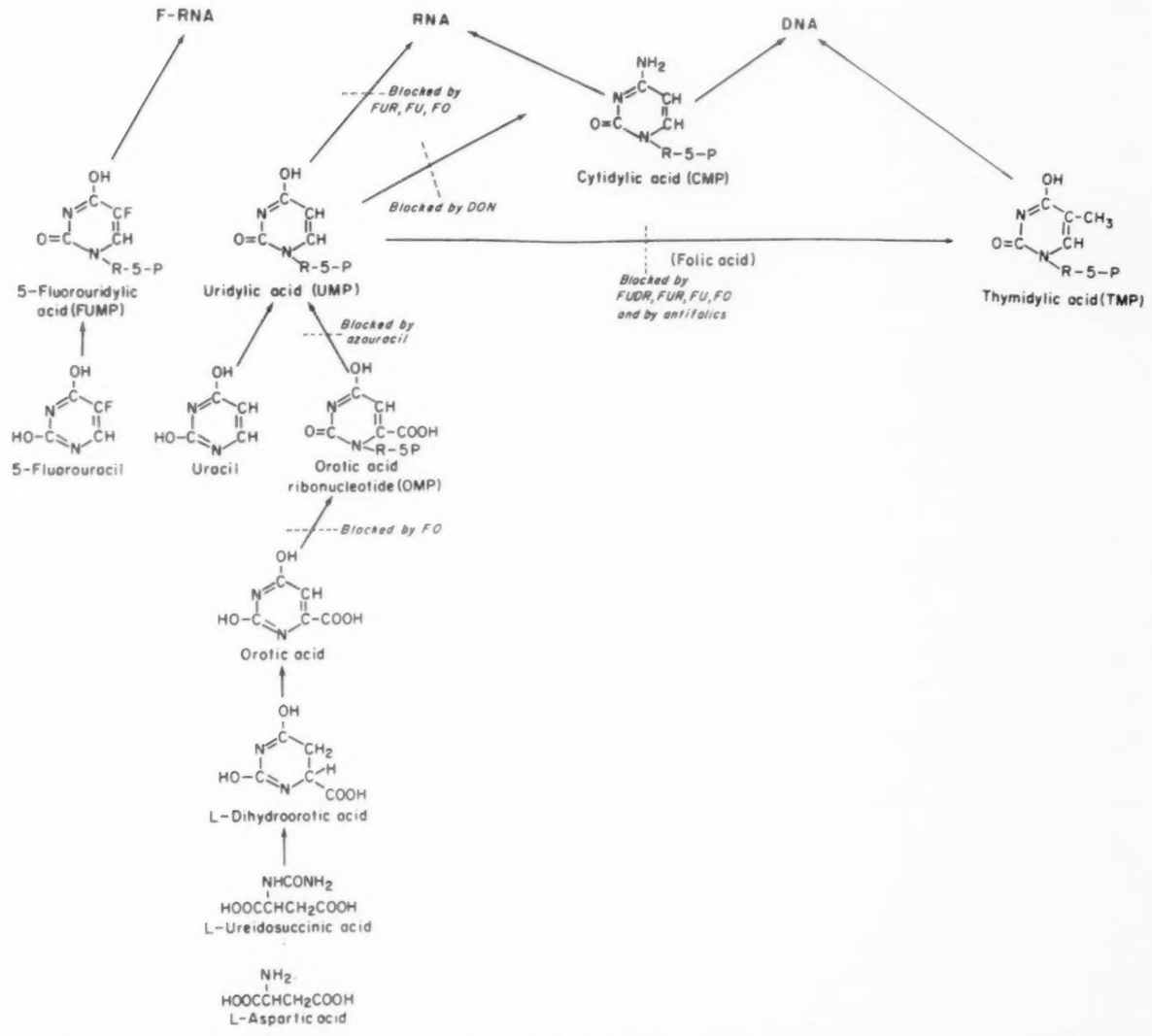


FIG. 4. Outline of the pathway of pyrimidine biosynthesis. The presumed sites of action of some antimetabolites affecting this system are indicated. The formation of "fraudulent" RNA from 5FU is shown in parallel with the incorporation of uracil into RNA, although it is not known to what extent the former pathway is responsible for the biologic activity of 5FU.

are not uncommon. There is some evidence of increased collateral sensitivity to amethopterin in transplanted mercaptopurine-resistant mouse leukemia [*24*], although the converse does not appear to be true. In adults with acute leukemia, who are usually unresponsive to therapy with folic acid antagonists, a few remissions have been observed with amethopterin in patients who have previously developed resistance to treatment with 6-mercaptopurine [*25*]. However, the number of cases observed to date is not sufficient to consider this a consistent pattern of increasing collateral sensitivity.

Glutamine Antagonists. O-diazoacetyl-L-serine (azaserine) and 6-diazo-5-oxo-L-norleucine (DON) were isolated from soil filtrates in the process of screening antibiotics [*26–28*]. These two compounds are effective against a similar spectrum of tumors and leukemias in mice [*29–33*]. Azaserine, the first of these substances to be isolated, was initially suspected of functioning as an antimetabolite because it was observed to inhibit the incorporation of C^{14}-labeled formate and glycine into nucleic acids of normal and leukemic tissues in mice [*34,35*]. This inhibition was shown, in an *in vitro*, cell-free pigeon liver system, to occur in the *de novo* synthesis of purines (Fig. 2) at the step in which

formylglycineamide ribonucleotide (FGAR) is aminated to yield formylglycineamidine ribonucleotide (FGAM) [*36*]. The same metabolic site of inhibition has been demonstrated in bacteria [*37*] and in the intact chicken [*38*] in which both azaserine and DON cause a profound inhibition of uric acid synthesis [*39*]. Glutamine serves as the donor of the amino group in the FGAR → FGAM reaction, and the inhibitory effect of azaserine and DON is due to their competition with glutamine for the enzyme concerned with the transfer. The inhibition can be partially prevented but not reversed by glutamine [*40,41*]. Studies with purines and purine precursors have been helpful in clarifying and amplifying knowledge of the mechanism of action of these compounds. Although azaserine and DON inhibit the incorporation of labeled formate and glycine into nucleic acid purines, they do not prevent the incorporation of labeled amino-imidazole carboxamide (AIC) or physiologic purines in mouse tissues or bacteria. Azaserine-induced growth inhibition in bacteria can be prevented with purines or AIC [*35,37*]. In the chick embryo, the characteristic teratogenic effects produced by azaserine and DON are prevented by AIC or purines without, however, modifying the lethality [*42*].*

In the intact chicken, nearly complete inhibition of uric acid synthesis is produced by the administration of median lethal doses of azaserine, with accumulation of the precursor, FGAR [*38,39*]. The biosynthetic mechanism recovers in twelve to eighteen hours but the animal may die of azaserine toxicity three or four days later. It appears, therefore, that in this species at least, there is either a discrepancy between purine biosynthesis and toxicity or quantitative differences between the ability of various tissues to recover from azaserine-induced inhibition of purine biosynthesis. Such differences in recovery time of various mouse tissues have been shown in *in vivo* studies [*44*].

Azaserine and DON inhibit many other reactions in which glutamine serves as an amino-group donor, among which are the conversion of 5-phosphoribosyl-pyrophosphate to 5-phosphoribosyl amine [*40*] (Fig. 2), the amination of uridylic acid to cytidylic acid [*45*] (Fig. 4) and a number of similar reactions in amino acid synthesis. These sites of action appear, however, to be quantitatively less significant than the inhibition of the FGAR → FGAM reaction.

As with the other antimetabolites, it has been possible to develop azaserine resistance in a previously responsive tumor in mice [*46*]. It was demonstrated that purine biosynthesis was inhibited by azaserine equally in the sensitive and resistant lines but that the resistant tumor recovered its ability to synthesize purines more rapidly than the sensitive one, and that the resistant tumor developed a greater ability to utilize preformed purines for nucleic acid synthesis during the period of azaserine inhibition. The azaserine-resistant tumor was cross-resistant to the related compound, DON, but not to 6-mercaptopurine, an antimetabolite of a different class.

In man, DON and azaserine have been shown to inhibit purine biosynthesis in certain circumstances, i.e., both compounds can prevent the increase in *de novo* synthesis of uric acid produced by the 2-substituted thiadiazoles [*47*] and there is evidence that these compounds may modify the increased production of uric acid seen in some cases of primary gout [*48*], although they have not been shown to inhibit synthesis of uric acid or other purines in normal human subjects. Despite this evidence in man, and the clear evidence in many other biologic systems of the ability of these compounds to block purine biosynthesis and in spite of their pronounced effects on the growth of transplanted leukemias and tumors in animals, neither compound has been found to exert an appreciable influence on any of the leukemias in man, either alone or in combination with other antimetabolites [*49,50*]. The reason for this is not clear. More studies of the metabolic pathways in man will be necessary to determine the biochemical mechanisms responsible for these discrepancies.

Purine Analogs. 6-Mercaptopurine (6-MP), the first of these compounds to become readily available, has been used much more widely than the related substances, 6-thioguanine and 6-chloropurine, which have similar mechanisms of action and therapeutic value. These compounds were synthesized [*51,52*] as part of a program to construct analogs of the known purine constituents of the nucleic acids. 6-MP was found to be effective in inhibiting bacterial

* It has been possible to produce improvement in patients with hypercalcemia and hypercalciuria due to osteolytic metastases from breast cancer with DON while protecting them from the oral toxicity of DON by the concurrent administration of adenine [*43*]. This suggests that, in this instance, it may be possible to divorce qualitatively the antitumor effects from the toxicity of the compound.

growth [*53*] and the growth of transplanted tumors and leukemias in mice [*24,54*]. These findings were followed quickly by the observation of their effectiveness in producing remission in acute leukemia in man [*55*] by a mechanism which appeared to be different from that of folic acid antagonists since it was possible to achieve remission in patients known to be resistant to amethopterin therapy.

The precise mechanism of action of 6-MP is still being investigated. It has been shown in bacteria that 6-MP inhibits the utilization of hypoxanthine for nucleic acid purines [*56*] and in tumor-bearing mice that 6-MP inhibits incorporation of formate-C^{14} into nucleic acids but does not inhibit the incorporation of labeled adenine [*57*]. The incorporation of hypoxanthine appeared to be inhibited by 6-MP in this system also, indicating that the principal site of action of the antimetabolite might be in the conversion of a hypoxanthine-containing compound to an adenine-containing compound. This has been confirmed and amplified by the finding that 6-MP is converted *in vivo* to the ribonucleotide which is the active form, and that this inhibits the conversion of inosinic acid to adenylosuccinic acid, the immediate precursor of adenylic acid [*58–60*]. (Fig. 2.) As with other agents, the mechanism is not unique for leukemic cells but is also capable of affecting the rapidly proliferating normal cells of bone marrow and intestinal epithelium. However, some strains of leukemic cells are at least quantitatively more susceptible than normal cells so that it is possible to achieve a high percentage of remissions in children with acute leukemia. Resistance to 6-MP eventually develops in man and has been studied in various experimental systems. It has been possible to develop 6-MP resistant lines of bacteria [*61,62*] and mouse leukemia [*24*]. In resistant lines, there are varying degrees of cross resistance to other purine analogs but not to other types of antimetabolites. Multiple mechanisms of resistance have been demonstrated. An important one appears to be due to the emergence of a mutant cell line which has lost the capacity to convert 6-MP to 6-MP ribonucleotide [*58,59,63–65*], a phenomenon which has been shown to occur in bacteria and in mammalian leukemic cells. Other strains of bacteria appear to have developed resistance because they have acquired the ability to synthesize purines *de novo* to a greater extent than the original sensitive strains [*59*].

Several other purine analogs have been synthesized and tested in animals and man. Some of these have shown no therapeutic effect at toxic doses, e.g., purine and 8-azaguanine. Others, such as 6-thioguanine and 6-chloropurine, have been found to be useful in about the same degree as 6-MP and have therefore not been widely used. Studies of 6-thioguanine [*66*] have indicated that its mechanism of action is similar to that of 6-MP. The ribonucleoside of thioguanine [*67*] has been found to be effective in a spectrum of leukemia in man similar to that of the other purine analogs but at a significantly smaller dosage when given orally [*68*]. This difference in dosage however, may only be due to differences in absorption, distribution or excretion rather than to a true metabolic difference.

Pyrimidine Analogs. This group of compounds has not been found to be useful in the management of clinical leukemia although brief remissions have been reported [*69*]. However, they are considered to be of sufficient interest to warrant discussion in this section because of their demonstrated influences on nucleic acid synthesis; some of them have been active in experimental leukemia. The results in the treatment of certain other non-leukemic neoplasms in man have confirmed their activity in inhibiting tumor growth.

The fluorinated pyrimidines represent another class of antimetabolites synthesized [*72*] because of the known role of uracil in nucleic acid synthesis. Prior work [*70,71*] had shown that certain rat tumors utilized uracil to a greater extent than the comparable normal tissues. 5-Fluorouracil (5FU) and other fluorinated pyrimidines have been found to be active against a number of transplanted tumors and leukemias in animals [*73–77*]. The mechanisms by which these compounds are thought to act metabolically are shown in Figure 4. 5FU has been shown to be incorporated into RNA but not into DNA of normal and tumor tissues of tumor-bearing animals and into the tumor RNA of a patient with metastatic carcinoma [*78*]. In the latter, the incorporation into tumor was greater than into surrounding skin, muscle, fat and liver. It is not known to what extent, if at all, the incorporation of 5FU into RNA is responsible for its antitumor effect. Probably the most important site of action of these compounds is in inhibiting the methylation of uridylic acid (UMP) (or deoxyuridylic acid, DUMP) to form thymidylic acid (TMP) [*79–81*], as shown by the

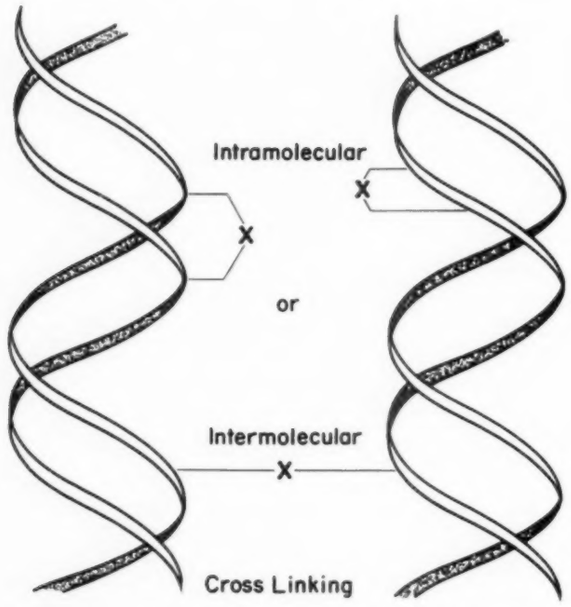


FIG. 5. Diagrammatic representation of the twin helical structure of DNA. The purine and pyrimidine ribonucleosides which connect the "strands" of phosphate are not shown. The various types of cross-linkages by polyfunctional alkylating agents are illustrated.

marked inhibition of incorporation of labeled uracil and orotic acid into DNA thymine but enhancement of incorporation of labeled thymidine. An additional site of action of fluorinated pyrimidines (except FUDR) is on the incorporation of uracil and orotic acid into RNA [*80,82*]. The conversion of orotic acid to orotidylic acid (OMP) and UMP has also been shown to be prevented by 5-fluoroorotic acid (5FO) but not by 5FU [*83*].

The specificity of these sites of action is illustrated by the failure of these compounds to prevent incorporation of labeled precursors into protein or nucleic acid purines while markedly inhibiting the synthesis of nucleic acid pyrimidines.

The brominated and iodinated analogs of uracil have been found to have growth-inhibitory activity in bacteria [*84*]. These compounds and the corresponding deoxyribonucleosides, bromodeoxyduridine (BrUDR) and iododeoxyuridine (IUDR), are incorporated into the DNA of bacteria [*85,86*] and mammalian cells in tissue culture [*87–89*]. BrUDR can substitute for thymine under some circumstances even though it can also be inhibitory. The growth relationships resulting from this dual activity have been well demonstrated in the sand-dollar embryo [*90*] (Echinarachnius parma), the development of which is inhibited in different ways and at different stages by FUDR and BrUDR. It is possible to protect the embryo from FUDR with either thymidine or BrUDR; however, if BrUDR is used as the protective agent developmental arrest occurs later, at a stage characteristic of BrUDR alone. The protective effect is not apparent in all systems, however, and in transplanted mouse leukemia the effect of FU or FUDR appears to be potentiated by BrUDR or IUDR as well as by 6-azathymine, a thymine analog which has shown no significant antileukemic effect when used alone [*91,92*]. Whether or not a better "therapeutic index" can be obtained by this type of combination is still under study in experimental tumors and leukemias and in patients with leukemia and other neoplastic diseases [*93*].

Another pyrimidine analog, 6-azauracil, has been found [*94*] to inhibit the *de novo* synthesis of pyrimidines by blocking the conversion of OMP to UMP. Clinical trials of this compound in acute leukemia have been undertaken but the results have been negative and neurologic toxicity was marked. The compound is not currently considered to have any clinical usefulness [*95*].

Polyfunctional Alkylating Agents. A polyfunctional alkylating agent can be defined as a compound possessing two or more end groups (alkyl groups) which may be either cyclic or unsaturated or can be converted to such forms. These unstable (reactive) end groups are capable of attaching to other molecules through an oxygen, nitrogen or sulfur atom [*96*]. Many such compounds are widely used in industrial chemistry. Their biologic interest is an outgrowth of work carried out by and for the Chemical Warfare Service during World War II and during that period much was learned of their effects [*97*]. Although it is possible (in appropriate concentrations) for these agents to react with many cellular constituents, it has been suggested [*98*] that at the concentrations found *in vivo* there is some preferential reaction with DNA. It may be that the reaction is not preferential but merely that the effects on DNA are more readily apparent. The influence on DNA is believed to be responsible for the principal effects occurring in dividing tissues such as leukemic and certain tumor cells, normal bone marrow and intestinal epithelium. Figure 5 demonstrates diagrammatically the manner in which the reactive groups of the alkylating agents attach to the

phosphate groups of the strands of DNA, producing various types of cross linkages and thereby "denaturing" or inactivating the DNA. Weakening of the DNA chain with cleavage at the point of attachment of the alkyl group may also occur, with resulting inactivation or change in the biologic properties of the DNA. However, cross linking is thought to be the more important of the two reactions. Although monofunctional alkylating agents (those possessing only a single reactive group) have some activity, it is of a smaller order of magnitude and no monofunctional agents have been developed which are clinically useful.

Chromatographic studies of DNA [*99,100*] isolated from the leukocytes of patients with chronic granulocytic leukemia have shown marked changes between the DNA of the untreated patient and that of the same patient following treatment with Myleran.® It is likely that these changes are due to cross linking and/or cleavage and that they are related to the accompanying biologic changes. Similar studies revealed differences between the DNA of normal human leukocytes and that from leukemic granulocytes, suggesting the existence of qualitative differences unrelated to treatment.

The mechanism of action of these compounds, which inactivate or destroy nucleic acids, is different from those of the antimetabolites discussed previously which affect growing cells by inhibiting various synthetic processes on the pathways to nucleic acids. This has been well demonstrated by comparative "sterilization" experiments employing mouse leukemic cells *in vivo* and *in vitro* [*101*]. It has been shown that exposure of the cells to nitrogen mustard or other alkylating agents in doses of 1 to 10 times the median lethal dose prevented the transmission of leukemia to normal mice whereas exposure to folic acid antagonists in doses up to 100 times the median lethal dose failed to prevent such transmission. Subsequent studies [*102*] with other alkylating agents and antimetabolites have consistently shown this difference between the two classes of compounds.

Figure 6 shows the formulas of the commonly used representatives of each major type of alkylating agent. It should be noted that the reactive groups of HN_2 (nitrogen mustard, Mustargen®), chlorambucil and Cytoxan® (cyclophosphamide) are identical in that all are β-chloroethyl amines, although the prosthetic groups are quite different. A different class of alkylating agents, the ethylene imines, includes triethylene melamine (TEM) and triethylene thio-phosphoramide (Thio-TEPA) which possess identical reactive groups with different prosthetic groups. Myleran (busulfan) is one of a series of sulfonoxy esters. Diepoxypiperazine is one of a series of basic epoxides with alkylating properties. All of these compounds have been found to be effective in a similar spectrum of leukemias and tumors in experimental animals [*103–105*] and man [*106–112*]. Modifications of the prosthetic groups have resulted in differences in side effects, solubility and stability but there is no evidence that any differences in therapeutic spectrum or "therapeutic index" have been produced by such alterations. Similarly, there is meager evidence that differences in reactive groups have produced changes in the specificity of alkylating agents for normal or neoplastic tissue in man. Experiments on the hematopoietic system of rats indicate that Myleran in that system specifically damages granulocytes, and that chlorambucil affects lymphocytes [*113*]. The clinical impression has therefore developed that Myleran is specific for granulocytic leukemia and chlorambucil for lymphocytic leukemia. However, a number of reports have indicated that Myleran can satisfactorily control diseases of the lymphatic system and chlorambucil can effectively control granulocytic leukemia [*114–116*]. A recent report [*117*] has suggested that in leukemic patients there may be quantitative differences in specificity for lymphatic or myeloid tissues of the β-chloroethyl amines versus the disulfonoxy esters. Specificity of action has also been suggested for Cytoxan (cyclophosphamide) on the basis of a higher phosphamidase activity of tumors than of most normal tissues; consequently the phosphamide linkage of Cytoxan (Fig. 5) might be expected to be split more readily in tumor tissue, liberating the reactive group in the desired intracellular location. To date, no evidence of such selectivity has been presented and the clinical data are as yet too sparse to warrant such an interpretation.* It has been suggested, also, that less thrombocytopenia may be produced by Cytoxan than by equivalent doses of other alkylating agents and this, too, is undergoing clinical evaluation.

Clear evidence of a preferential effect of any

* Cytoxan sterilizes leukemic mouse cells *in vivo* but not *in vitro* [*102*]; this may indicate an *in vivo* change or "activation" of this specific alkylating agent.

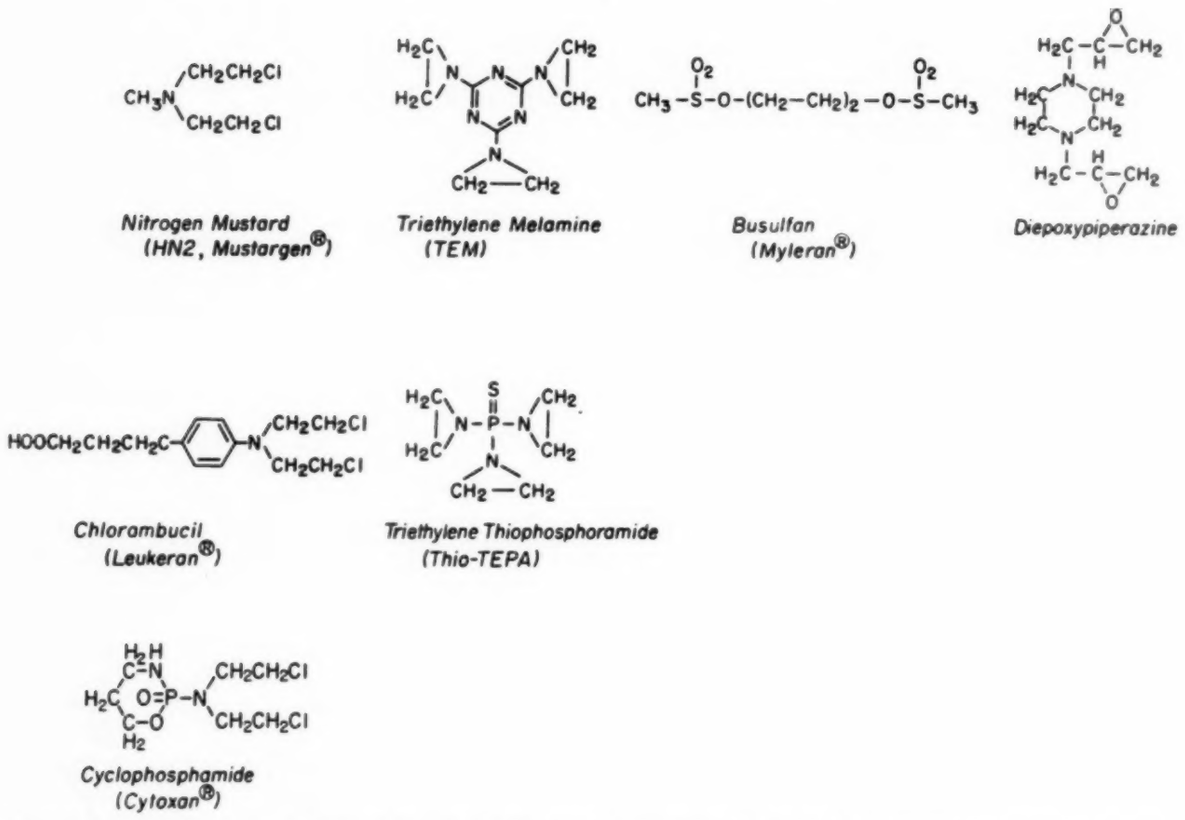


FIG. 6. Structural formulas of representatives of major types of polyfunctional alkylating agents: β-chloroethylamines, ethylene imines, disulfonoxy esters and diepoxides.

one type of alkylating agent on a specific type of leukemia, tumor or normal tissue would be of major importance, since it would indicate the possibility of synthesizing agents which could selectively affect a given tissue. At present, however, such evidence is only inferential.

Adrenal Cortical Hormones. Cortisone and related compounds and their derivatives have come to play an important role in the management of some leukemias. Nevertheless, their mechanism of action is not well understood, although a number of relevant facts has been developed which may lead to a better understanding of the biochemical mechanisms involved in the production of remissions in acute leukemia and improvement in chronic lymphocytic leukemia.

Early in the era of the adrenal hormones it was observed [*118*] that they had the ability to produce lysis of lymphocytes. Although this lytic phenomenon is characteristic of both normal and leukemic lymphocytes, much larger amounts of hormone are required to lyse leukemic than normal cells. *In vitro* studies [*119*] have shown that human leukemic lymphocytes are capable of catabolizing C^{14}-labeled cortisol to a much greater extent than can normal cells. Leukemic granulocytes catabolized cortisol differently. Soon after the availability of these substances it was recognized [*120,121*] that they could inhibit tumor growth in experimental animals, and soon after that they were found to produce improvement in chronic lymphocytic leukemia and remissions in acute leukemia in man [*122,123*]. Presumably it is their lytic effect on specific cell types that is responsible for the remissions in acute leukemia as well as the improvement sometimes seen in chronic lymphocytic leukemia. As with other useful agents in these diseases, resistance eventually develops, usually more quickly than antimetabolite-resistance. A clear understanding of the mechanisms of resistance to these compounds will have to await more clarification of the biochemical mechanisms involved in their antileukemic activity.

*Miscellaneous Compounds.** The ability of *colchicine* to produce arrest of the metaphase in cell division has been known for at least seventy years and there is evidence that it was used locally in the treatment of "tumors" more than 2,000 years ago [*124*]. However, because of the severe gastrointestinal toxicity which it produces colchicine has not been useful in the treatment of leukemia, although it is capable of producing bone marrow depression. The isolation [*125*] of the alkaloid, demecolcine (desacetylmethylcolchicine) from the parent plant, *Colchicum autumnale* (the autumn crocus), provided a preparation relatively free of these side effects. This compound has been used in the treatment of chronic granulocytic leukemia [*126*]. Demecolcine and the related alkaloid, n-desacetylthiocolchicine [*127*], like colchicine, produce mitotic arrest in the metaphase in growing plants and in mammalian cells in tissue culture; it is presumably this property which is responsible for their antileukemic effects. The biochemical mechanisms which produce this phenomenon have not been defined.

The action of *urethan* (ethyl carbamate), a compound of simple molecular structure, is poorly understood. It has anesthetic properties as well as the ability to depress granulopoiesis. Because of this latter property it has been used in the treatment of chronic granulocytic leukemia, although it has now been largely replaced by the newer antimetabolites and alkylating agents. Studies in animals have shown [*128*] that its antitumor activity can be inhibited by thymidine or cytidine but not by uracil or uridine, implying interference with the utilization of pyrimidines for DNA synthesis but this has not been completely clarified.

Potassium arsenite (Fowler's solution) is of interest chiefly because it was the first systemic agent found to have an effect on a neoplastic disease [*129,130*]. It appears to be a general cell poison, interfering with respiratory activity of leukemic and normal leukocytes [*131*].

Benzene can regularly depress the bone marrow and for that reason has been used in the treatment of chronic granulocytic leukemia [*132*]. It is no longer employed for this purpose.

Sulfapyridine [*133*] has been reported to reduce the leukocyte count in chronic lymphocytic leukemia and, paradoxically, *para-aminobenzoic acid* [*134*] given in large doses has been found to reduce the count in chronic granulocytic leukemia, in spite of the fact that neither is theoretically implicated in the metabolism of mammalian cells. Except for occasional idiosyncrasies, neither appears toxic to the normal human hematopoietic system. The mechanism of effect on chronic leukemia in these few reports has not been defined.

The regular bone marrow depressants which may produce clinical improvement in the chronic leukemias should be distinguished from the long list of compounds which have been shown to produce depression of specific or multiple cellular elements in the blood by means of a sensitivity or antigen-antibody reaction. Among this latter group are compounds such as aminopyrine [*135*] and quinidine [*136,137*], which have been shown to affect leukocytes and thrombocytes respectively, and chloramphenicol which has been thought on clinical grounds to produce generalized marrow aplasia due to sensitivity, although a precise demonstration of the sensitivity phenomenon has not been made. It has been shown [*138*] that relatively large doses of chloramphenicol can produce reversible marrow depression in nonsensitive human subjects and it is possible that both regular depression at high dose levels and sensitivity reactions at lower doses may occur with this or other substances. Agents which depress hematopoiesis by means of sensitivity reactions cannot be considered predictable or useful in the treatment of human leukemia.

COMMENTS

In considering the manner in which various chemical or physical agents modify the course of leukemia in man, it is necessary to conceive of the leukemias as a group of diseases which differ widely from one another in many respects. It is apparent that the various types of leukemia differ in the morphology of the cells involved, in the ways in which they influence the host, in their natural history and evolution, and in the ways in which they respond to treatment.

Some of the relationships of the various types of leukemic cells to one another and to certain normal cells in terms of their responsiveness to chemotherapeutic agents are shown diagrammatically in Figure 7. This emphasizes that leukemic cells may be metabolically different,

* The compounds included in this section are termed "miscellaneous" not because they do not belong in the other categories but because their mechanisms of action are not sufficiently understood to warrant categorizing them.

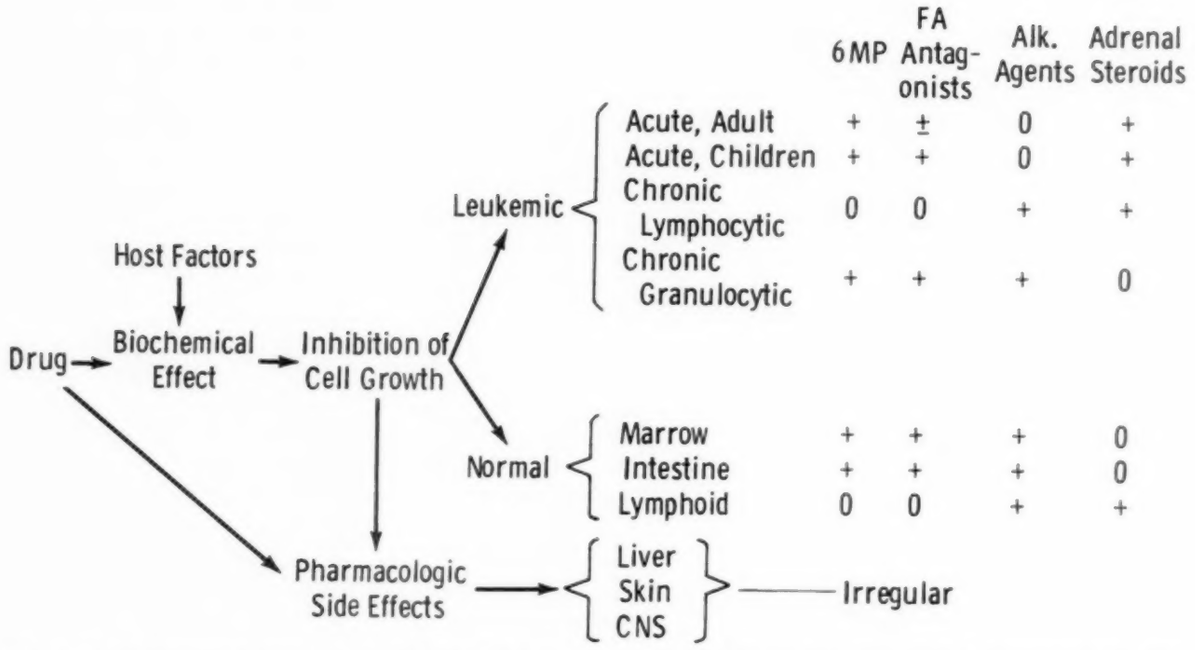


FIG. 7. The differences in susceptibility of various cell types to a given agent can be represented diagrammatically. For instance, in response to 6-MP, normal marrow and intestine and chronic granulocytic leukemia are regularly susceptible, stem cells in children with acute leukemia are frequently susceptible, morphologically similar stem cells in adults with acute leukemia are infrequently susceptible, and chronic lymphatic leukemia is not susceptible. It is apparent that in relation to 6-MP, the folic acid antagonists and the alkylating agents, there is a uniformity of response of normal marrow and intestinal epithelium which is closely paralleled by the response of chronic granulocytic leukemia but not by other leukemic cells. The susceptibility to adrenal steroids follows a different pattern, chronic granulocytic leukemia again responding in a manner similar to that of the normal tissues.

even if morphologically similar. Successful treatment of a leukemia will depend upon influencing a metabolic pathway which is present and vulnerable in that particular type, and perhaps even in the particular case.

An example of the relation of therapeutic responsiveness to possible metabolic differences can be seen in the contrast between chronic granulocytic leukemia, which responds well to 6-MP and at least transiently to amethopterin, and chronic lymphocytic leukemia which does not respond at all to these compounds. This difference may be explained by studies [*139*] which suggest that either the life span of leukemic lymphocytes is extremely long or that they are able to reutilize large polynucleotide fragments for nucleic acid synthesis. Leukemic granulocytes are shorter-lived or are not capable of polynucleotide reutilization, and must therefore utilize the pathway of *de novo* purine biosynthesis, which can be blocked by antimetabolites.

It should be clear that what is meant by "response" is not the same for each type of disease. In chronic granulocytic leukemia, for example, which is characterized by exuberant proliferation of myeloid elements in the bone marrow and spleen, response is merely the quantitative suppression of this proliferation, allowing the resumption of normal erythropoiesis and temporary restoration of clinical health. In chronic lymphocytic leukemia, response probably represents a qualitative change in the disease, with correction of hemolytic anemia, restoration of immune function of the serum globulins, and (probably least important) reduction in the leukocyte count. In the acute leukemias response or remission clearly represents a qualitative change, i.e., destruction or suppression of abnormal cells and repopulation of the marrow and peripheral blood with normal cellular elements. An example of these qualitative differences in response is seen in the effects of alkylating agents on the chronic leukemias versus acute leukemia. Treatment of the chronic leukemias with one of the polyfunctional alkylating agents regularly produces a fall in leukocytes along with improvement in other hematologic and clinical parameters. In acute leukemia these

compounds can depress the leukocyte count equally well but this is rarely accompanied by hematologic or clinical evidence of improvement.

Differences in susceptibility to chemotherapeutic agents are not related only to differences in morphology; there is a markedly better rate of response in children with acute leukemia than in adults with morphologically similar acute leukemia. It is not yet clear whether the lessened responsiveness in adults is due to host factors or to true metabolic differences in the cells of the two age groups.

The failure of leukemia in man to respond to agents such as azaserine and DON which are effective in inhibiting the growth of numerous transplanted animal tumors and leukemias, and which have been shown in multiple biologic systems to be potent inhibitors of purine biosynthesis, suggests that there may be important metabolic differences between leukemias in man and the various animal systems which have been studied.

The biochemical mechanisms which are influenced by the agents which have been discussed are the normal mechanisms and pathways retained by the neoplastic cells and it has therefore been possible to demonstrate effects of these compounds on a variety of normal tissues as well as on tumors and leukemias. By blocking a common metabolic pathway, cell growth may be inhibited in many tissues; this may be most apparent in the rapidly growing neoplastic tissues, resulting in suppression of the manifestations of their neoplastic qualities. When growth resumes, due to resistance or to withdrawal of therapy, the neoplastic qualities again become apparent but this does not imply that it is specifically those qualities which have been blocked by therapy. Although, as was pointed out earlier, no qualitative biochemical or metabolic differences between normal and neoplastic cells have been defined, it is reasonable to infer from the differences in behavior that such differences may exist. Effective utilization of substances now available and the development of more effective agents will depend on more accurate definition of the biochemical abnormalities in these disorders and on more precise characterization in man of the mechanisms by which resistance develops in a previously sensitive population of cells.

Acknowledgment: I am indebted to my colleagues at the Sloan-Kettering Institute, particularly Drs. D. A. Karnofsky, R. R. Ellison, J. H. Burchenal, M. E. Balis, A. Bendich and J. J. Fox for many discussions which were helpful in the preparation of this review.

REFERENCES

1. Davidson, J. N. The Biochemistry of the Nucleic Acids. London, 1952. Methuen & Co., Ltd.
2. Brown, D. M. and Todd, A. R. *J. Chem. Soc.*, 1: 52, 1952.
3. Markham, R. and Smith, J. D. *Biochem. J.*, 52: 552, 1952.
4. Watson, J. D. and Crick, F. H. C. *Nature, London*, 171: 737, 1953.
5. Wooley, D. W. A Study of Antimetabolites. New York, 1952. John Wiley & Sons.
6. Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., Jr. and Wolff, J. A. *New England J. Med.*, 238: 787, 1948.
7. Skipper, H. E., Mitchell, J. H., Jr., Bennett, L. L., Jr., Newton, M. A., Simpson, L. and Eidson, M. *Cancer Res.*, 11: 145, 1951.
8. Nichol, C. A. and Welch, A. D. *Proc. Soc. Exper. Biol. & Med.*, 74: 52, 1950.
9. Nichol, C. A. and Welch, A. D. *Proc. Soc. Exper. Biol. & Med.*, 74: 403, 1950.
10. Greenberg, G. R. *Fed. Proc.*, 13: 745, 1954.
11. Goldthwait, D. A. *Proc. Soc. Exper. Biol. & Med.*, 86: 842, 1954.
12. Goldthwait, D. A. In: The Leukemias: Etiology, Pathophysiology and Treatment. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press.
13. Huennekens, F. M., Whiteley, H. R. and Osborn, M. J. *J. Cell. & Comp. Physiol.* (supp. 1), 54: 109, 1959.
14. Burchenal, J. H., Kushida, M. N., Johnston, S. F. and Cremer, M. A. *Proc. Soc. Exper. Biol. & Med.*, 71: 559, 1949.
15. Burchenal, J. H., Babcock, G. M., Broquist, H. P. and Jukes, T. H. *Proc. Soc. Exper. Biol. & Med.*, 74: 735, 1950.
16. Burchenal, J. H. and Kingsley-Pillers, E. M. *J. Clin. Invest.*, 30: 631, 1951.
17. Sullivan, R. D., Miller, E. and Sykes, M. P. *Cancer*, 12: 1248, 1959.
18. Goldthwait, D. A. and Bendich, A. *J. Biol. Chem.*, 196: 841, 1952.
19. Naber, E. C., Snell, E. E. and Cravens, W. W. *Proc. Soc. Exper. Biol. & Med.*, 81: 20, 1952.
20. Burchenal, J. H., Robinson, E., Johnston, S. F. and Kushida, M. N. *Science*, 111: 116, 1950.
21. Law, L. W. and Boyle, P. J. *Proc. Soc. Exper. Biol. & Med.*, 74: 599, 1950.
22. Broquist, H. P., Kohler, A. R., Hutchison, D. J. and Burchenal, J. H. *J. Biol. Chem.*, 202: 59, 1953.
23. Hutchison, D. J. and Burchenal, J. H. In: Ciba Foundation Symposium: Chemistry and Biology of Pteridines. Edited by Wolstenholme, G. E. W., Cameron, M. P. London, 1954. J. & A. Churchill.
24. Law, L. W. *Proc. Soc. Exper. Biol. & Med.*, 84: 409, 1953.
25. Karnofsky, D. A. and Ellison, R. R. Proceedings

of the Seventh Congress of International Society of Hematology, in press.

26. Bartz, Q. R., Elder, C. C., Frohardt, R. P., Fusari, S. A., Haskell, T. H., Johannessen, D. W. and Ryder, A. *Nature, London*, 173: 72, 1954.
27. Ehrlich, J., Coffey, G. L., Fisher, M. W., Hillegas, A. B., Kohberger, D. L., Machamer, H. E., Rightsel, W. A. and Roegner, F. R. (Abstract.) *Am. Chem. Soc., Dallas Meet.*, p. 12-M, 1956.
28. Dion, H. W., Fusari, S. A., Jakubowski, Z. L., Zora, J. G. and Bartz, Q. R. (Abstract.) *Am. Chem. Soc., Dallas Meet.*, p. 13-M, 1956.
29. Stock, C. C., Reilly, H. C., Buckley, S. M., Clarke, D. A. and Rhoads, C. P. *Nature, London*, 173: 71, 1954.
30. Burchenal, J. H., Murphy, M. L., Yuceoglu, M. and Horsfall, M. *Proc. Am. A. Cancer Res.*, 1: 7, 1954.
31. Burchenal, J. H. and Dagg, M. K. *Proc. Am. A. Cancer Res.*, 2: 97, 1956.
32. Clarke, D. A., Reilly, H. C. and Stock, C. C. *Proc. Am. A. Cancer Res.*, 2: 100, 1956.
33. Sugiura, K. and Schmid, M. S. *Proc. Am. A. Cancer Res.*, 2: 151, 1956.
34. Skipper, H. E., Bennett, L. L., Jr. and Schabel, F. M., Jr. *Fed. Proc.*, 13: 298, 1954.
35. Bennett, L. L., Jr., Schabel, F. M., Jr. and Skipper, H. E. *Arch. Biochem.*, 64: 423, 1956.
36. Hartman, S. C., Levenberg, B. and Buchanan, J. M. *J. Am. Chem. Soc.*, 77: 501, 1955.
37. Tomisek, A. J., Kelly, H. J. and Skipper, H. E. *Arch. Biochem.*, 64: 437, 1956.
38. Krakoff, I. H., Wheeler, G. R. and Karnofsky, D. A. Unpublished observations.
39. Krakoff, I. H. and Karnofsky, D. A. *Am. J. Physiol.*, 195: 244, 1958.
40. Levenberg, B., Melnick, I. and Buchanan, J. M. *J. Biol. Chem.*, 225: 163, 1957.
41. Buchanan, J. M., Levenberg, B., Melnick, I. and Hartman, S. C. In: The Leukemias: Etiology, Pathophysiology and Treatment. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press.
42. Dagg, C. P. and Karnofsky, D. A. *Fed. Proc.*, 15: 238, 1956.
43. Magill, G. B., Myers, W. P. L., Reilly, H. C., Putnam, R. C., Magill, J. W., Sykes, M. P., Escher, G. C., Karnofsky, D. A. and Burchenal, J. H. *Cancer*, 10: 1138, 1957.
44. Moore, E. C. and LePage, G. A. *Cancer Res.*, 17: 804, 1957.
45. Eidinoff, M. L., Knoll, J. E., Marano, B. and Cheong, L. *Cancer Res.*, 18: 105, 1958.
46. Sartorelli, A. C. and LePage, G. A. *Cancer Res.*, 18: 457, 1958.
47. Krakoff, I. H. and Balis, M. E. *J. Clin. Invest.*, 38: 907, 1959.
48. Grayzel, A. I. and Seegmiller, J. E. *J. Clin. Invest.*, 38: 1008, 1959.
49. Burchenal, J. H., Murphy, M. L. and Tan, C. T. C. *Pediatrics*, 18: 643, 1956.
50. Heyn, R. M., Brubaker, C. A., Burchenal, J. H., Cramblett, H. G. and Wolff, J. A. *Blood*, in press.
51. Elion, G. B., Burgi, E. and Hitchings, G. H. *J. Am. Chem. Soc.*, 74: 411, 1952.
52. Hitchings, G. H. and Elion, G. B. *Ann. New York Acad. Sc.*, 60: 195, 1954.
53. Elion, G. B., Hitchings, G. H. and Vanderwerff, H. *J. Biol. Chem.*, 192: 505, 1951.
54. Clarke, D. A., Philips, F. S., Sternberg, S. S., Stock, C. C., Elion, G. B. and Hitchings, G. H. *Cancer Res.*, 13: 593, 1953.
55. Burchenal, J. H., Murphy, M. L., Ellison, R. R., Sykes, M. P., Tan, T. C., Leone, L. A., Karnofsky, D. A., Craver, L. F., Dargeon, H. W. and Rhoads, C. P. *Blood*, 8: 965, 1953.
56. Elion, G. B., Singer, S. and Hitchings, G. H. *J. Biol. Chem.*, 204: 35, 1953.
57. Skipper, H. E. *Ann. New York Acad. Sc.*, 60: 315, 1954.
58. Balis, M. E., Levin, D. H., Brown, G. B., Elion, G. B., Nathan, H. C. and Hitchings, G. H. *Arch. Biochem.*, 71: 358, 1957.
59. Balis, M. E., Hylin, V., Coultas, M. K. and Hutchison, D. J. *Cancer Res.*, 18: 440, 1958.
60. Salser, J. S., Hutchison, D. J. and Balis, M. E. *J. Biol. Chem.*, in press.
61. Elion, G. B., Singer, S. and Hitchings, G. H. *Ann. New York Acad. Sc.*, 60: 200, 1954.
62. Hutchison, D. J. *Ann. New York Acad. Sc.*, 60: 212, 1954.
63. Brockman, R. W. and Hutchison, D. J. *Fed. Proc.*, 18: 197, 1959.
64. Salser, J. S. and Balis, M. E. *Fed. Proc.*, 18: 315, 1959.
65. Brockman, R. W., Sparks, C., Simpson, M. S. and Skipper, H. E. *Biochem. Pharm.*, 2: 77, 1959.
66. Sartorelli, A. C. and LePage, G. A. *Cancer Res.*, 13: 1329, 1958.
67. Fox, J. J., Wempen, I., Hampton, A. and Doerr, I. L. *J. Am. Chem. Soc.*, 80: 1669, 1958.
68. Krakoff, I. H., Ellison, R. R. and Tan, C. T. C. *Proc. Am. A. Cancer Res.*, 3: 34, 1959.
69. Murphy, M. L., Ellison, R. R., Aguila, F. S., Sullivan, R., Li, M. C. and Burchenal, J. H. Cancer chemotherapy reports, USPHS No. 2, p. 12, March 1959.
70. Rutman, R. J., Cantarow, A. and Paschkis, K. E. *Cancer Res.*, 14: 119, 1954.
71. Heidelberger, C., Liebman, K. C., Harbers, E. and Bhargava, P. M. *Cancer Res.*, 17: 399, 1957.
72. Duschinsky, R., Pleven, E. and Heidelberger, C. *J. Am. Chem. Soc.*, 79: 4559, 1957.
73. Heidelberger, C., Chaudhuri, N. K., Danneberg, P. B., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R. J., Pleven, E. and Scheiner, J. *Nature, London*, 179: 663, 1957.
74. Heidelberger, C., Griesbach, L., Montag, B. J., Mooren, D. and Cruz, O. *Cancer Res.*, 18: 305, 1958.
75. Burchenal, J. H., Holmberg, E., Wiegand, L. and Hemphill, S. *Proc. Am. A. Cancer Res.*, 2: 285, 1958.
76. Law, L. *Proc. Am. A. Cancer Res.*, 2: 318, 1958.
77. Liebling, M. E. and Humphreys, S. R. *Proc. Am. A. Cancer Res.*, 2: 321, 1958.
78. Chaudhuri, N. K., Montag, B. J. and Heidelberger, C. *Cancer Res.*, 18: 318, 1958.

79. Danneberg, P. B., Montag, B. J. and Heidelberger, C. *Cancer Res.*, 18: 329, 1958.
80. Bosch, L., Harbers, E. and Heidelberger, C. *Cancer Res.*, 18: 335, 1958.
81. Rich, M. A., Bolaffi, J. L., Knoll, J. E., Cheong, L. and Eidinoff, M. L. *Cancer Res.*, 18: 730, 1958.
82. Eidinoff, M. L., Knoll, J. E. and Klein, D. *Arch Biochem.*, 71: 274, 1957.
83. Stone, J. E. and Potter, V. R. *Cancer Res.*, 17: 800, 1957.
84. Hitchings, G. H., Elion, G. B., Falco, E. A., Russell, P. B. and VanderWerff, H. *Ann. New York Acad. Sc.*, 52: 1318, 1950.
85. Dunn, T. B. and Smith, J. D. *Nature, London*, 174: 305, 1954.
86. Zamenhof, S. and Griboff, G. *Nature, London*, 174: 306, 1954.
87. Eidinoff, M. L., Cheong, L. and Rich, M. A. *Science*, 129: 1550, 1959.
88. Eidinoff, M. L., Knoll, J. and Marano, B. J. *Proc. Am. A. Cancer Res.*, 2: 198, 1957.
89. Eidinoff, M. L., Knoll, J. E., Marano, B. J. and Klein, D. *Cancer Res.*, 19: 738, 1959.
90. Karnofsky, D. A. and Basch, R. S. *J. Biophys. & Biochem. Cytol.*, 7: 61, 1960.
91. Burchenal, J. H. and Oettgen, H. F. Cancer chemotherapy reports, USPHS No. 2, p. 16, March 1959.
92. Burchenal, J. H. and Oettgen, H. F. Cancer chemotherapy reports, USPHS, in press.
93. Young, C. W., Ellison, R. R., Sullivan, R. D., Levick, S. N., Kaufman, R., Miller, E., Woldow, I., Escher, G., Li, M. C., Karnofsky, D. A. and Burchenal, J. H. Cancer chemotherapy reports, USPHS, in press.
94. Handschumacher, R. E. and Pasternak, C. A. *Biochim. et Biophys. acta*, 30: 451, 1958.
95. Frei, E., Freireich, E. J., Holland, J. F., Pinkel, D., Sachs, M. S., Rothberg, H., Shnider, B. I., Stabenau, J. R., Taylor, R. J. and Zubrod, C. G. Program of the Seventh International Cancer Congress, p. 317, 1958.
96. Price, C. C. *Ann. New York Acad. Sc.*, 68: 663, 1958.
97. Gilman, A. and Philips, F. S. *Science*, 103: 409, 1946.
98. Stacey, K. A., Cobb, M., Cousens, S. F. and Alexander, P. *Ann. New York Acad. Sc.*, 68: 682, 1958.
99. Bendich, A., DiMayorca, G. P., Rosenkranz, H. S., Bianchessi, M. and Polli, E. E. *Haemat. latina* (supp. 2), p. 34, 1959.
100. Bendich, A., DiMayorca, G. P., Rosenkranz, H. S., Bianchessi, M. and Polli, E. E. *J. Nat'l. Cancer Inst.*, in press.
101. Burchenal, J. H., Cremer, M. A., Williams, B. S. and Armstrong, R. A. *Cancer Res.*, 11: 700, 1951.
102. Burchenal, J. H., Oettgen, H. F., Reppert, J. and Coley, V. *Proc. Am. A. Cancer Res.*, in press.
103. Burchenal, J. H., Lester, R. A., Riley, J. B. and Rhoads, C. P. *Cancer*, 1: 399, 1948.
104. Burchenal, J. H., Burchenal, J. R. and Johnston, S. F. *Cancer*, 4: 353, 1951.
105. Stock, C. C. and Sugiura, K. *Ann. New York Acad. Sc.*, 68: 853, 1958.
106. Rhoads, C. P. *J. A. M. A.*, 131: 656, 1946.
107. Galton, D. A. G., Israels, L. G., Nabarro, J. D. N. and Till, M. *Brit. M. J.*, 2: 1172, 1955.
108. Karnofsky, D. A., Burchenal, J. H., Armistead, G. C., Southam, C. M., Bernstein, J. L., Craver, L. F. and Rhoads, C. P. *Arch. Int. Med.*, 87: 477, 1951.
109. Galton, D. A. G. *Lancet*, 264: 208, 1953.
110. Shay, H., Zarafonetis, C., Smith, N., Woldow, I. and Sun, D. C. H. *Arch. Int. Med.*, 92: 628, 1953.
111. Krakoff, I. H., Miller, D. G., Karnofsky, D. A., Burchenal, J. H., Diamond, H. D. and Craver, L. F. Program of the Seventh International Cancer Congress, p. 294, 1958.
112. Coggins, R. R., Ravdin, R. G. and Eisman, S. H. Cancer chemotherapy reports, USPHS No. 3, p. 9, June, 1959.
113. Elson, L. A. *Brit. J. Haemat.*, 1: 104, 1955.
114. Sykes, M. P., Philips, F. S. and Karnofsky, D. A. *M. Clin. North America*, 40: 837, 1956.
115. Galton, D. A. G. In: Advances in Cancer Research. Edited by Greenstein, J. P. and Haddow, A. New York, 1956. Academic Press.
116. Krakoff, I. H., Karnofsky, D. A. and Burchenal, J. H. *J. A. M. A.*, 166: 629, 1958.
117. Rundles, R. W., Grizzle, J., Bell, W. N., Corley, C. C., Fronmeyer, W. B., Jr., Greenberg, B. G., Huguley, C. M., Jr., James, G. W., Jones, R., Jr., Larsen, W. E., Loeb, V., Leone, L. A., Palmer, J. G., Riser, W. H., Jr. and Wilson, S. J. *Am. J. Med.*, 27: 424, 1959.
118. Dougherty, T. F. and White, A. *Endocrinology*, 35: 1, 1944.
119. Dougherty, T. F. In: The Leukemias: Etiology, Pathophysiology and Treatment. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press.
120. Heilman, F. R. and Kendall, E. C. *Endocrinology*, 34: 416, 1944.
121. Murphy, J. B. and Sturm, E. *Science*, 99: 303, 1944.
122. Pearson, O. H., Eliel, L. P., Rawson, R. W., Dobriner, K. and Rhoads, C. P. *Cancer*, 2: 943, 1949.
123. Pearson, O. H., Eliel, L. P. and Talbot, T. R., Jr. *Bull. New York Acad. Med.*, 26: 235, 1950.
124. Eigsti, O. J. and Dustin, P. Colchicine in Agriculture, Medicine, Biology and Chemistry. Ames, 1955. Iowa State College Press.
125. Santavy, F. and Reichstein, T. *Helvet. chim. acta.*, 33: 1606, 1950.
126. Moeschlin, S., Meyer, H. and Lichtman, A. In: Ciba Foundation Symposium on Leukaemia Research. Edited by Wolstenholme, G. E. W. and Cameron, M. P. Boston, 1954. Little, Brown & Co.
127. Huguenin, R., Truhaut, R. and Saracino, R. *Bull. Cancer*, 42: 308, 1955.
128. Elion, G. B., Bieber, S., Nathan, H. and Hitchings, G. H. *Cancer Res.*, 18: 802, 1958.
129. Lissauer. *Berl. Klin. Wchnschr.*, 2: 403, 1865.
130. Karnofsky, D. A. In: Pharmacology of Medicine, 2nd ed. Edited by Drill, V. A. New York, 1958. McGraw-Hill.
131. Warren, C. O. *Am. J. Physiol.*, 139: 719, 1943.
132. Von Koranyi, A. *Berl. Klin. Wchnschr.*, 49: 1357, 1912.

133. Livingston, K. E. and Moore, R. D. *New England J. Med.*, 223: 975, 1940.
134. Zarafonetis, C. J. D., Andrews, G. A., Meyers, M. C. and Bethell, F. H. *Blood*, 3: 780, 1948.
135. Moeschlin, S. and Wagner, K. *Acta haemat.*, 8: 29, 1952.
136. Hirsch, E. O. and Dameshek, W. *Am. J. Med.*, 9: 828, 1950.
137. Larson, R. K. *Blood*, 8: 16, 1953.
138. Krakoff, I. H., Karnofsky, D. A. and Burchenal, J. H. *New England J. Med.*, 253: 7, 1955.
139. Hamilton, L. D. In: The Leukemias: Etiology, Pathophysiology and Treatment. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press.
140. Karnofsky, D. A. *Cancer Res.*, 16: 684, 1956.

The Anemia of Leukemia*

STANLEY B. TROUP, M.D., SCOTT N. SWISHER, M.D. *and* LAWRENCE E. YOUNG, M.D.
Rochester, New York

ANEMIA in the leukemic patient is so common that it may be considered an almost inevitable complication of the disease [*1–4*]. The multiple interacting mechanisms leading to the development of anemia in the various forms of leukemia have been the subject of intensive study in many laboratories, especially during the past two decades. This study examines these mechanisms from a primarily clinical point of view and with particular reference to hemolytic processes.

Historic. More than brief mention of the historic aspects of anemia in leukemia is beyond the intended scope of this review; this subject has been well reviewed elsewhere [*5,6*]. A few early contributions can be cited to bring some of the modern views of this problem into perspective. In 1878, several years before Ehrlich described the staining technics that permitted detailed microscopic examination of blood, Neumann [*7*] had noticed the presence of nucleated red cells in the blood of certain patients with leukemia. This observation cast doubt upon the concept prevalent at that time that red cells evolved from white cells. In addition to suggesting a dichotomy in the production of blood cells, Neumann also implied a defect in the production of red cells to account for the anemia in leukemia. The following year Gowers [*8*], although still subscribing to the view that red cells took origin from lymphocytes, postulated that impaired production *and* increased destruction of red cells might contribute to the anemia of leukemic patients. In the ensuing years reports appeared offering data in support of either increased red cell destruction [*5,9,10*] or impaired production [*11*] as the principal pathogenetic mechanism leading to anemia.

General Pathogenetic Mechanisms of Anemia in Leukemia. Until recently it was assumed that leukemic infiltration in the bone marrow of affected patients uniformly limited the production of red cells. The advent of radioisotopic technics has afforded opportunity to measure production and destruction of red cells *in vivo* with greater ease, and much of what is now known of red cell kinetics in these diseases has been contributed during the past decade [*5,12–16*]. The pathogenesis of anemia in the leukemic patient can now be viewed as a dynamic process. No longer can one cling to the mechanistic view that leukemic cells displace erythroid elements in the marrow and "crowd" them out of existence. The earliest data that seriously weakened this concept were histologic observations presented by Jaffé [*17,18*]. He demonstrated that despite active proliferation of leukemic cells in the marrow, the red cell elements could be *quantitatively* normal or even increased in certain patients. In some patients dense leukemic infiltration of the marrow and paucity of erythroid cells may indeed be observed, but a physical displacement of erythroid cells is nevertheless inadequate to explain the pathogenesis of anemia in most cases.

The development of anemia in the leukemic patient is a consequence of varying combinations of (1) decreased erythropoiesis with normal or increased red cell destruction or loss, or (2) normal or increased erythropoiesis with increased red cell destruction or loss.

Decreased erythropoiesis may be a consequence of hypoplasia of bone marrow [*19–23*], inhibition by metabolic competition [*15,24,25*], antileukemic therapy, dense leukemic infiltration or processes yet unknown. Shortening of red cell life span may be due to the production of defective red cells or to the effects of extracorpuscular factors. Autoimmune hemolytic anemia, which is a frequent complication of

* From the Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. Studies reported in this paper were supported by a contract with the Research and Development Division, Office of the Surgeon General, Department of the Army (DA-49-007-MD-632), a grant from the National Institutes of Health, National Institute of Arthritis and Metabolic Diseases, United States Public Health Service (A-537) and the David Smalline Chapter, International Leukemia Foundation, Inc.

chronic lymphocytic leukemia, provides the best known example of an extracorpuscular hemolytic mechanism. Other hemolytic mechanisms, not yet defined, probably exist.

Hyporegenerative Anemia with Hypoplastic Marrow in Leukemia. Hypoplasia of the bone marrow is now recognized with increasing frequency as a herald of more typical acute leukemia. In such cases leukopenia and thrombocytopenia are usually noted in addition to anemia, and frequently it is the thrombocytopenia with attendant bleeding that prompts the patient to seek medical care. The diagnosis of leukemia must be made with caution under these circumstances, for other causes of hypoplasia of the marrow may be responsible for similar clinical pictures. In the patient with hypoplasia of bone marrow due to leukemia, some diagnostic clues may be found. Careful study of the peripheral blood smears, especially smears of the concentrated "buffy coat" of sedimented nucleated cells, often reveals nucleated red blood cells as well as occasional abnormal or immature white cells. Morphologic abnormalities of the red cells produced in such a hypoplastic leukemic or "preleukemic" state are frequently impressive. Properly prepared and stained peripheral blood films frequently reveal significant variation in size and shape, and prominent basophilic stippling of the red cells. In our experience, morphologic abnormalities of the red cells are less frequently observed in patients who prove ultimately to have idiopathic or toxic hypoplasia of bone marrow.

Patients in whom leukemia appears following exposure to ionizing radiation may also pass through a period of anemia associated with hypoplasia of bone marrow [*26,27*]. A report on one such patient seen in this clinic is presented here.

J. A. S. (SMH No. 288914), a sixty-three year old white male dentist, first sought medical care in 1956 because of six months' of fatigue and several weeks of easy bruising. His appetite was impaired but there had been no weight loss. He had no other symptoms of consequence. Six years previously his left testicle had been removed because of a seminoma. The local area and contiguous lymph nodes were then radiated with 4,000 r. The patient had practiced dentistry for forty years and reported with some pride that he had been the first dentist in his community to employ x-rays for diagnostic purposes. He had used his original equipment for twenty-six years.

On initial examination he was a pale, well nourished, elderly man with purpuric areas over both legs. There was no enlargement of the lymph nodes, liver or spleen. The left testicle was absent. The remainder of the examination was within normal limits.

The hematocrit was 22 per cent, the white blood cell count was 2,500 per cu. mm. The blood smear revealed moderate thrombocytopenia, considerable variation in size and shape of the red cells, and a normal differential leukocyte count except for 2 per cent myeloblasts and 3 nucleated red cells per 100 white cells. The leukocyte alkaline phosphatase activity was in the high normal range [*28*]. Needle aspiration of the bone marrow yielded few marrow elements. Surgical marrow specimens were obtained from two separate sites, and both showed marked hypoplasia. (Fig. 1A.)

Blood transfusion was the sole therapy for five months. Infusion of chromium^{51}-labelled normal, serologically compatible red cells revealed 50 per cent of radioactivity lost in twenty-three days, a finding indicative of only slight shortening of red cell survival.

After five months of relative comfort the patient experienced an abrupt onset of fever, drenching night sweats and marked anorexia. The liver and spleen became moderately enlarged. The white blood cell count rose to 12,000 per cu. mm., virtually all of which were pathologic myeloblasts. The bone marrow was densely cellular and consisted chiefly of abnormal myeloblasts similar to those in the peripheral blood. Despite transfusion and the administration of 6-mercaptopurine and corticosteroids, the patient died two weeks following the sudden change in clinical course. Figure 1B is a photomicrograph of the bone marrow specimen obtained at autopsy.

The anemia manifest initially by this patient was probably a consequence of the hypoplasia of bone marrow and decreased production of red cells. The persistent reticulocytopenia and nearly normal survival of normal donor red cells are in keeping with this formulation. The presence of normoblastemia, anisocytosis and poikilocytosis, and occasional abnormal and immature white cells in the peripheral blood, suggested at the outset, despite the hypoplasia of bone marrow, that the patient had leukemia rather than idiopathic or secondary hypoplasia of bone marrow.

Hyporegenerative Anemia with Normally Cellular or Hyperplastic Marrow in Leukemia. Anemia of surprisingly long duration, accompanied by normally active or hyperplastic bone marrow, also may antedate the appearance of a more typical leukemic picture. In recent years many examples of this type of onset of leukemia have been recorded [*21,22,29–32*]. A patient with long-standing anemia previously reported on from this clinic [*33*] was studied over a period of six-

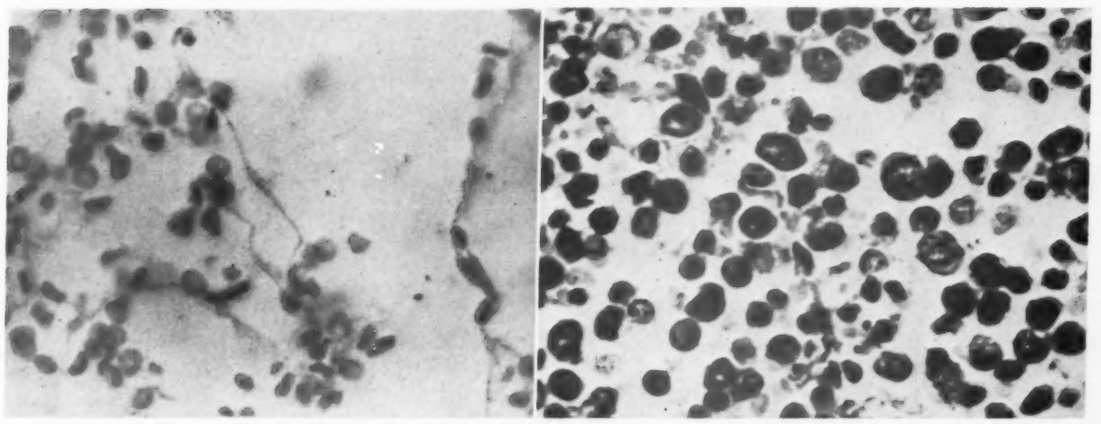


FIG. 1. A, section of bone marrow from patient J. S., obtained during stage of leukopenia and anemia, demonstrating marked hypoplasia. B, postmortem section of marrow from same patient showing leukemic infiltration.

teen months before the diagnosis of chronic myelocytic leukemia was established. A striking feature of this case was the morphologic abnormality of the the red cells. Figure 2A is a photomicrograph of the peripheral blood smear showing the red cells during the preleukemic period. Figure 3 shows a graph of the osmotic fragility of the freshly drawn red cells of this patient, and demonstrates increased osmotic resistance of the abnormally thin erythrocytes.

Similar changes in osmotic fragility of red cells have been observed in a number of more typical cases of acute leukemia from this clinic. Figure 2B is a photomicrograph of the blood smear from such a patient (Case 17 in Table 1). Other patients studied in this clinic have had similar but less prolonged preleukemic periods of anemia accompanied by impressive morphologic abnormalities of the red cells. Figure 2C, a photomicrograph of the peripheral blood from another such patient, illustrates the variation in size and shape of the red cells during the initial period of observation when the bone marrow was normally cellular. In this patient a typical, acute myeloblastic leukemia developed after more than sixteen months of unexplained anemia.

The pathologic mechanisms resulting in anemia in this preleukemic group of patients are not clear. Critical studies of the rates of red cell production and destruction are not available in adequate numbers of these patients. Several features suggest that increased destruction of red cells plays some role. Elevation of the indirect-reacting bilirubin fraction, chronic reticulocytosis and increase in fecal urobilinogen [*21,29*] have all been observed in such patients. While such data are suggestive of hemolysis, Nathan and Berlin [*34*] have cited evidence that reticulocytosis in certain patients with acute leukemia is related to premature release of red cells by the marrow and does not indicate an increase in erythropoiesis. Baldini et al. [*25*] have demonstrated that the increase in fecal excretion of urobilinogen noted in patients with erythroleukemia may be due to diversion of heme pigment from sources other than destroyed red cells, as is known to occur in other diseases [*35*].

Mechanisms of Anemia in Acute Leukemia and Chronic Myelocytic Leukemia. Mechanisms responsible for anemia in patients with more typical manifestations of leukemia have been studied in detail. These processes differ greatly in importance in various groups of patients with leukemia, and from patient to patient within any group. Wetherley-Mein et al. [*36*] studied red cell kinetics in patients with acute leukemia and in patients with chronic leukemia before and during therapeutically-induced remissions of the leukemic process. They demonstrated that repair of anemia in these patients occurred through a combination of effects, one of which was increased erythropoiesis. Prior to treatment of their seven leukemic patients showing increased destruction of red cells the marrow failed to compensate completely by increased regeneration of red cells. The central role of impaired production of red cells in the pathogenesis of anemia in many leukemic patients is emphasized by their studies.

The shortening of red cell life span in patients with either acute or chronic leukemia

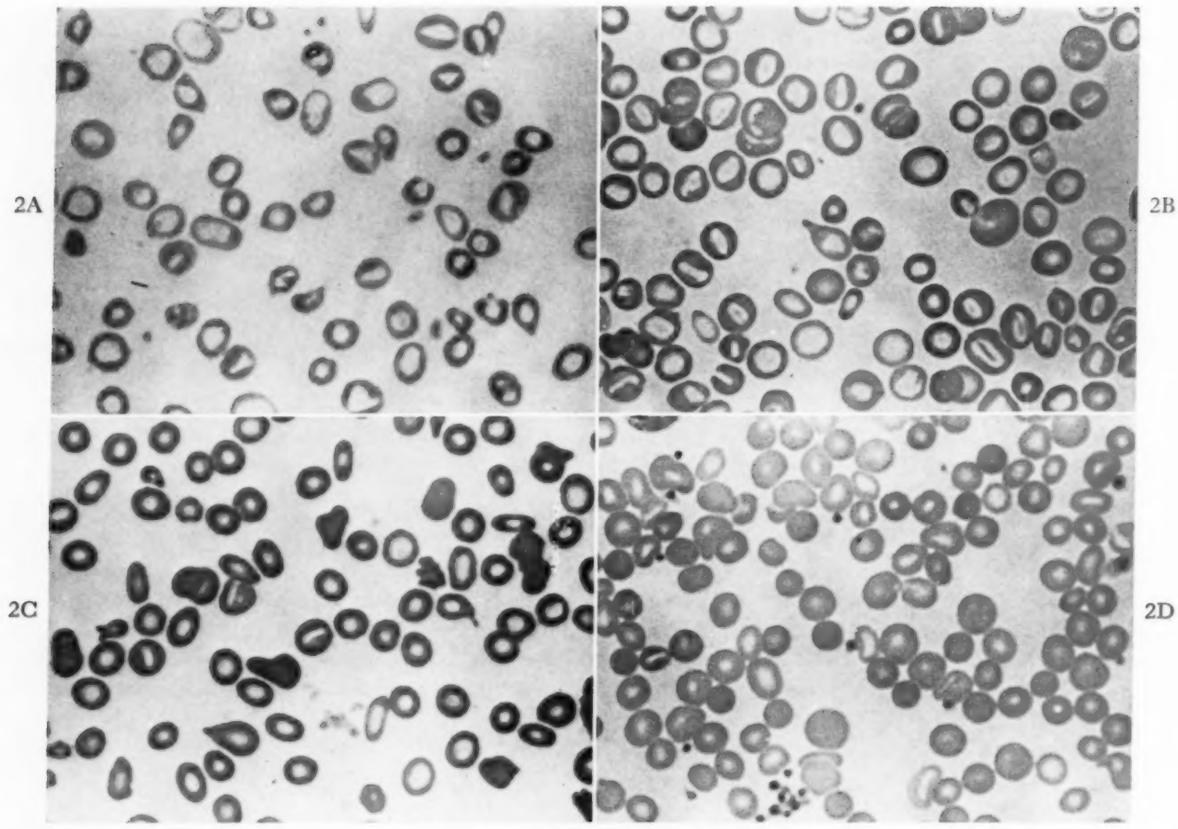


FIG. 2. A, photomicrograph of blood smear from patient E. B. during preleukemic state, showing variation in size and shape of red cells. The osmotic fragility of this patient's red cells is graphed in Figure 3. B, photomicrograph of blood smear from a patient with acute leukemia. The red cells show marked variation in size and moderate poikilocytosis. This patient's red cells demonstrated increased osmotic resistance and abnormal autohemolysis. (Case 17, Table 1.) C, photomicrograph of blood smear from a patient with acute leukemia displaying well marked poikilocytosis. (Case 12, Table 1.) D, photomicrograph of blood smear from patient T. O. with myeloid metaplasia, displaying anisocytosis and spherocytosis. The osmotic fragility of this patient's red cells is graphed in Figure 3.

may involve the patient's own red cells or compatible normal donated cells, as shown by Nathan and Berlin [*34*] and by Wetherley-Mein et al. [*36*], in patients with acute forms of leukemia. Acute, severe hemolysis occurs most frequently in patients with chronic lymphocytic leukemia complicated by autoimmune hemolytic anemia.

Although the dynamics of red cell production and destruction have been well described in many patients with leukemia, the inability of the leukemic marrow to provide adequate erythropoiesis is poorly understood. Metabolic competition of several kinds may exist between the developing red cells and pathologic white cells, as suggested by Berlin et al. [*15*]. The presence of normal or increased numbers of normoblastic red cell precursors in the marrow does not always indicate that the production of red cells is proceeding at a proportional rate. Baldini et al. [*25*] suggest this possibility to explain the disparity between the appearance of the marrow and erythropoietic rate in the di Guglielmo syndrome of erythroleukemia, in which megaloblast-like patterns of erythropoiesis may occasionally be seen. Megaloblast-like changes in red cell precursors may also be encountered in the marrow of patients with other forms of leukemia [*24,37*]. Administration of vitamin B_{12}, folic acid or liver extract to these patients does not increase measurably the rate of erythropoiesis. Although some patients with acute leukemia have erythroid precursors in normal or near normal numbers in the marrow, many show nearly complete replacement of the marrow by pathologic blast forms. Patients with leukemia may also suffer inhibition of erythropoiesis because of a complicating infection [*38*].

Spontaneous In Vitro Lysis of Red Cells from Patients with Leukemia. Little is known about

TABLE 1

RESULTS OF AUTOHEMOLYSIS MEASUREMENTS IN PATIENTS WITH LEUKEMIA AND MYELOID METAPLASIA

Case No.	Diagnosis	Poikilocytosis	Per cent Hemolysis		
			No Additive	Glucose Added	Adenosine Added
1	Acute leukemia	+++	1.8	0.7	0.8
2	Acute leukemia	.....	0.2	0.2	0.1
3	Acute leukemia	++	0.7	2.5	0.1
4	Acute leukemia	+	0.8	1.1	0.3
5	Acute leukemia	++	3.6	1.4	0.7
6	Acute leukemia	++++	9.2	1.9	0.7
7	Acute leukemia	+	5.9	0.7	0.3
8	Acute leukemia	++	8.3	0.2	1.6
9	Acute leukemia	++	6.1	0.4	3.9
10	Acute leukemia	++	8.2	2.0	3.1
11	Acute leukemia	++	5.3	1.3	2.0
12	Acute leukemia	+	11.5	1.5	7.5
13	Acute leukemia	+	5.5	1.5	2.0
14	Acute leukemia	+	6.0	0.5	4.0
15	Acute leukemia	+	8.0	2.0	3.0
16	Acute leukemia	+	6.0	0.5	4.0
17	Acute leukemia	++	11.2	9.0	5.1
18	Acute leukemia	++++	21.0	4.6	9.8
19	Subacute lymphocytic leukemia	+	6.7	0.9	1.2
20	Subacute monocytic leukemia	++	4.2	2.1	0.9
21	Subacute myelocytic leukemia	++	7.1	16.1	2.2
22	Subacute myelocytic leukemia	+++	6.7	6.6	9.9
23	Subacute myelocytic leukemia	+++	3.0	5.1	1.5
24	Subacute myelocytic leukemia	+	3.5	0.5	0.5
25	Subacute myelocytic leukemia	+	6.1	0.4	3.8
	Chronic myelocytic leukemia (10 patients)	.....	0.8 (mean) 0.2–2.7 (range)	.....	.....
	Myeloid metaplasia (22 patients)	.....	3.4 (mean) 0.6–8.5 (range)	4.4 (mean) 0.3–25.1 (range)	1.7 (mean) 0.2–13.4 (range)
	Normal donors	.....	1.5 (mean) ±1.0 (S.D.)* 0–5.5 (range)	0.0 (mean) ±0.1 (S.D.) 0–0.5 (range)	0.5 (mean) ±0.2 (S.D.) 0–1.0 (range)

* Standard deviation.

abnormalities of metabolism in the red blood cells in leukemia. One approach to the exploration of such abnormalities is the measurement of lysis of red cells from patients with leukemia during periods of incubation of sterile defibrinated blood with and without added glucose or purine nucleosides. Methods used in our laboratory for measurement of *in vitro* autohemolysis and results obtained in preliminary studies on blood from patients with leukemia are described in an earlier report [*39*].

The observations to date on autohemolysis are regarded only as exploratory and do not differentiate between abnormalities of the red cells and possible hemolytic effects mediated by the leukocytes, platelets or serum. In its simplest form, the procedure consists of incubating aliquots of blood at 37°C. for forty-eight hours,

with resuspension of the cells in the serum at twenty-four hours. At forty-eight hours the cells and serum are separated by centrifugation and the proportion of the total hemoglobin released from the red cells in each aliquot is determined. Prior to incubation glucose is added to one aliquot to provide a concentration of about 30 mM, and adenosine and inosine are added separately to other aliquots to provide concentrations of either 4.5 or 18 mM.

Measurements of autohemolysis in tests of this type by Selwyn and Dacie [*40*] and in our laboratory support the conclusion that lysis above the normal range is strong presumptive evidence of a hemolytic disorder. However, a normal rate of autohemolysis does not exclude the possibility of a hemolytic process in any given case. In hereditary spherocytosis the uniformly marked autohemolysis has been shown to be related to abnormalities of the red cells and to be correlated with the degree of spherocytosis. In patients with leukemia the abnormalities responsible for accelerated autohemolysis in these crude tests have not yet been defined, but nevertheless the results are of interest.

Table 1 presents representative measurements of autohemolysis in blood from thirty-five patients with leukemia and twenty-two patients with myeloid metaplasia. Fifteen of the twenty-five patients with acute or subacute leukemia, examined prior to any remission, yielded autohemolysis figures above the normal range and without apparent relationship to the degree of poikilocytosis. Only two patients (Cases 12 and 21) in this group showed spherocytosis. Addition of glucose caused marked inhibition of hemolysis in most of these cases, the notable exceptions being three cases of subacute myelocytic leukemia. These results are in general accord with the observations of Crosby and Benjamin [*41*] who demonstrated a capacity of plasma from patients with acute leukemia to lyse both autogenous and normal red cells. One of the features of the *in vitro* hemolytic system described by these authors was its inhibition by glucose.

The results of autohemolysis determinations in blood specimens from ten patients with chronic myelocytic leukemia were within normal limits, and in these cases the appearance of the red cells in the blood smears was unremarkable. In myeloid metaplasia, on the other hand, autohemolysis was significantly increased in some cases, notably those in which spherocytosis was well marked. In nearly all examinations of this group hemolysis was accelerated by addition of glucose, sometimes to a striking degree.

The effect of glucose on autohemolysis may in part be due to a reduction in pH as a result of the formation of lactic acid during incubation of the blood. Acidification of blood with lactic, citric or hydrochloric acid caused marked acceleration of lysis of red cells from two of our most frequently examined patients with myeloid metaplasia and persistent spherocytosis. (A representative osmotic fragility curve obtained with blood from one of these patients, T. O., is shown in Figure 3. A photomicrograph of this patient's blood smear is shown in Figure 2D.) These results are in striking contrast to those obtained in hereditary spherocytosis in which addition of glucose or acidification of the blood invariably reduces autohemolysis.

Addition of adenosine prior to incubation caused significant reduction in autohemolysis in nearly all cases of leukemia and myeloid metaplasia; the reduction was more marked with the larger of the two amounts of adenosine employed. Addition of inosine gave variable results, probably due in part to lack of buffering effect, as contrasted with adenosine which yields ammonia on incubation and thus maintains a relatively high pH in the incubated blood.

The results of these preliminary studies on autohemolysis in leukemia seem to point to multiple hemolytic mechanisms, some involving abnormalities of the red cells, others involving plasma factors, some inhibited and some potentiated by addition of glucose or by reduction of pH of the incubated blood.

Mechanisms of Anemia in Chronic Lymphocytic Leukemia. Hemolytic anemia of the autoimmune type, characterized by a positive direct red cell antiglobulin (Coombs') test, occurs not infrequently in patients with chronic lymphocytic leukemia. During the years 1947 to 1959, ten cases of autoimmune hemolytic anemia were encountered in this clinic among seventy-seven cases of chronic lymphocytic leukemia under observation. A similar incidence has been found by others [*6,42*]. This type of hemolytic anemia is also encountered with about the same frequency in patients with lymphosarcoma and reticulum cell sarcoma. The total incidence of anemia developing by various mechanisms in patients with chronic lymphocytic leukemia is much greater. In our experience, severe anemia usually occurs late in the course of chronic lymphocytic leukemia. Autoimmune hemolytic

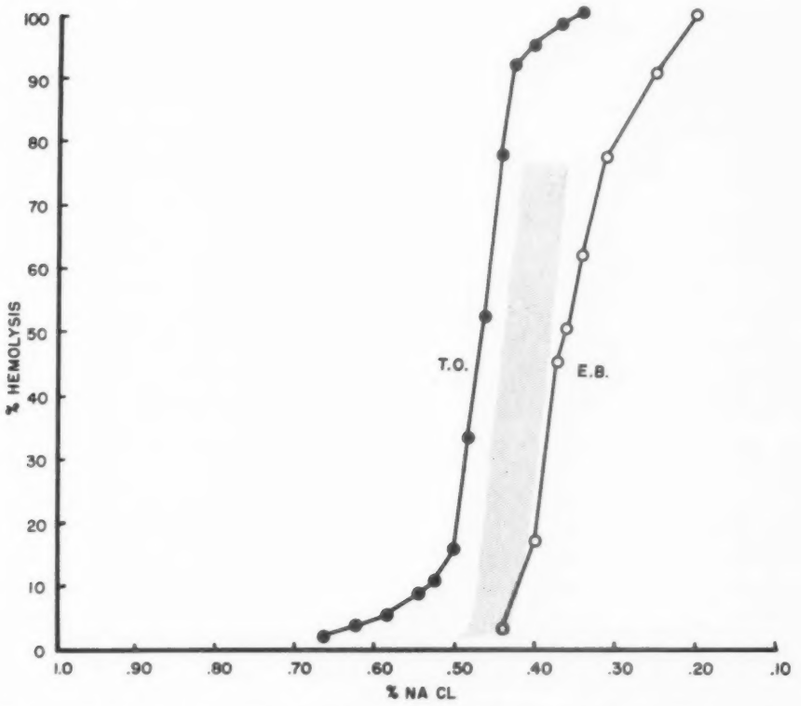


FIG. 3. Graph of the osmotic fragility of freshly drawn red cells. The normal range is indicated by the stippled area. Patient E. B., in a preleukemic state had red cells showing persistently decreased osmotic fragility. (A photomicrograph of this patient's blood smear is shown in Figure 2A.) Patient T. O. with myeloid metaplasia and persistent spherocytosis has had red cells showing increased osmotic fragility throughout a ten-year period of observation. The curve shown is representative. (A photomicrograph of this patient's blood smear is shown in Figure 2D.)

anemia, however, may occur at any time during the illness. The finding of relatively severe anemia early in the patient's course should prompt the physician to consider the presence of a hemolytic state.

In the experiences summarized by Dameshek and Gunz [*6*] about half the patients with chronic lymphocytic leukemia and autoimmune hemolytic anemia had evidence of hemolysis at the time leukemia was first recognized. In the remainder, months to many years intervened between diagnosis of leukemia and the apparent onset of hemolysis. Their experience parallels our own in this regard. Serial red cell antiglobulin tests in patients with lymphocytic leukemia and lymphoma have been of great value in the early detection and prompt management of this condition.

The clinical pictures and the management of symptomatic autoimmune hemolytic anemia have been well reviewed by a number of authors [*42–45*]. Only those aspects especially pertinent to the course of this disorder in patients with chronic lymphocytic leukemia will be discussed here. The severity of the hemolytic state may vary widely. Some patients may have only minor shortening of the red cell life span, which is detectable solely by direct measurement with Cr^{51}-labelled red cells, either autogenous or normal donor cells. Other patients experience sudden onset of hemolysis which may be rapidly fatal unless properly treated. Although the reaction to the antiglobulin test usually remains positive, at least for several months and in some cases for a number of years, the rate of red cell destruction may fluctuate widely and for reasons that are not presently known [*46–48*]. These fluctuations in hemolytic activity are not well correlated with objective changes in the course of the underlying leukemia. As the leukemia progresses, anemia may become more severe, frequently as a result of a decrease in compensatory erythropoiesis. Remission or moderation of hemolysis sometimes follows antileukemic therapy with radiation or alkylating drugs. However, Rosenthal et al. [*45*] have sug-

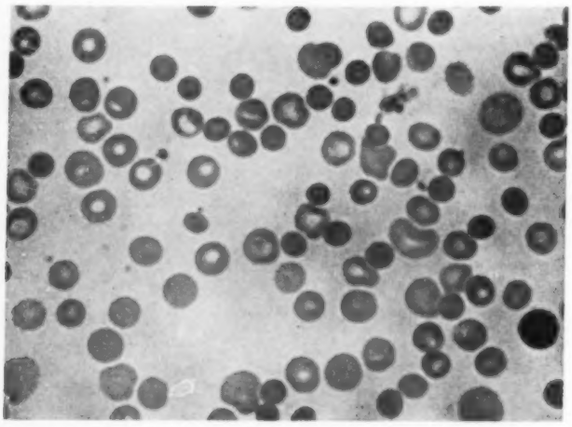


FIG. 4. Photomicrograph of the blood smear of a patient with chronic lymphocytic leukemia and active autoimmune hemolytic anemia. Numerous spherocytes are present. The larger, thinner-appearing cells are polychromatophilic and stain as reticulocytes.

gested that the onset of hemolysis may follow such therapy. More commonly, the hemolytic process requires treatment with corticosteroid or ACTH at times when the leukemic process appears to be stable and not to require therapy.

Spherocytosis and increase in osmotic fragility of the red cells may be observed during periods of active hemolysis, as in other types of autoimmune hemolytic disease. When the hemolytic state is in remission or only slightly active, red cell morphology is usually normal. With active hemolysis, normoblastemia, marked anisocytosis, with many macrocytic, polychromatophilic red cells, poikilocytosis of varying degree and punctate basophilia are also frequently observed. Figure 4, a photomicrograph of a blood smear from such a patient, illustrates some of these abnormalities. Bone marrow examinations reveal a normoblastic pattern of erythropoiesis. If the normoblastic series is markedly hyperplastic, it may become somewhat macrocytic with "loosening" of the nuclear chromatin pattern. These changes, common to many severe hemolytic disorders, have been mistaken for those of a true megaloblastic anemia. The mechanisms by which such changes occur in the normoblastic cells of the marrow are not yet clearly defined.

During periods of active red cell destruction the erythrocytes of patients with chronic lymphocytic leukemia and autoimmune hemolytic anemia may also undergo lysis somewhat more rapidly than normal in the type of autohemolysis test previously described. Table II

TABLE II

RESULTS OF AUTOHEMOLYSIS MEASUREMENTS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA COMPLICATED BY AUTOIMMUNE HEMOLYTIC DISEASE

Case No.	Hemolytic State	Spherocytosis	Per cent Hemolysis		
			No Additive	Glucose Added	Adenosine Added
1	Active	Slight	2.0	0.1	0.3
1	In remission	None	0.8	0.2	...
2	Active	Moderate	3.6	4.9	0.8
2	In partial remission	Slight	1.6	3.2	0.6
3	Active	Slight	2.3	1.9	0.4
4	Active	Slight	1.1	0.8	0.3
5	Active	None	0.3	0.2	...

summarizes typical results of autohemolysis tests in five patients. Addition of glucose to the blood in Case 2 resulted in slight but significant increase in autohemolysis and had minimal inhibitory effect on autohemolysis in the other cases. Addition of adenosine usually caused a decrease in autohemolysis.

Serologic Problems in Autoimmune Hemolytic Anemia. The frequency with which red cell-coating autoantibodies are detected in patients with chronic lymphocytic leukemia depends in part upon the tests used for their detection and in particular upon the type of antiglobulin serum employed. Although the characteristics of the autoantibodies of the entire group of autoimmune hemolytic disorders are quite varied, the antibodies detected in patients with lymphocytic leukemia or lymphoma are almost always of the "warm type," having maximal activity at temperatures near 37°C. Autoantibodies more active at lower temperatures (4 to 10°C.) have been observed in cases of symptomatic autoimmune hemolytic anemia associated with malignant reticuloendothelial disorders [*43,49*] but these are not common and have not been encountered in this laboratory. Both gamma globulins and non-gamma globulins are encountered as red cell sensitizing autoantibodies, the former predominating [*43*]. Other abnormalities of the plasma proteins, such as hypergammaglobulinemia, cryoglobulinemia and macroglobulinemia, are found in these patients but as yet there is no evidence that abnormal proteins of these types play a role in the mechanisms of increased destruction of red cells.

Not all patients with malignant lymphocytic diseases and overt hemolytic anemia have demonstrable evidence of red cell sensitization

by an autoantibody [*42,47*]. In some, autoantibodies not detectable by conventional serologic technics may be present. Substances that appear to react *in vivo* as isoantibodies, but are undetectable serologically, have been described in man [*50–52*] and in the dog [*53*], and it seems possible that a similar "order" of autoantibodies may exist. The usual kinds of red cell sensitization may not be detected if the antiglobulin serum employed in these tests is inadequate in potency in either the antigamma or antinongamma globulin components. Unfortunately, many commercially available antiglobulin reagents are unable to detect non-gamma types of red cell sensitization since they may be produced specifically for use in blood grouping and crossmatching reactions. Even in these latter types of reactions ability to detect non-gamma red cell sensitization is important. The need for more precise definition and standardization of the reactivity of antiglobulin reagents is evident.

Prozones rarely may account for failure to detect red cell sensitization by the antiglobulin technic. It is necessary to employ several dilutions of antiglobulin serum, in the range of 1:10 to 1:1000, to eliminate this possibility, particularly when the serum is potent. More commonly, positive direct antiglobulin reactions will be missed if the reaction is not read immediately upon adding the antiglobulin serum to the washed red cells. In our laboratory these tests are carried out in small tubes, which are immediately and gently centrifuged and examined for agglutination. The tubes are then allowed to stand for fifteen minutes at room temperature, are recentrifuged and re-examined. The mechanism whereby agglutination under these circumstances occasionally disappears after a short period of incubation is not known, and no special significance can be given to its occurrence.

It seems likely that processes capable of greatly accelerating red cell destruction, but not mediated by autoantibodies, may develop in patients with most varieties of leukemia. Overt, severe hemolytic states of this type are not commonly encountered in cases of chronic lymphocytic leukemia, but minor degrees of shortening of red cell life span are common. The latter situation usually develops late in the course of the illness and may be manifest principally by excessive requirements for transfusion, a need for transfusion exceeding that which might be expected if there were complete lack of erythropoiesis. In adults a requirement of more than 500 ml. of transfused red cells (1,000 ml. whole blood) every ten days to two weeks usually can be considered as excessive in the absence of significant blood loss. The usual clinical and laboratory indications of increased destruction of red cells may not be found. Hyperbilirubinemia, reticulocytosis, normoblastic hyperplasia of the marrow and changes in appearance or in osmotic fragility of the red cells may all be lacking. The mechanisms of this subtle type of red cell destruction, which may be common to a wide range of leukemias and other malignant reticuloendothelial disorders, are poorly understood. It is likely that a number of independent mechanisms may be involved.

Treatment of Autoimmune Hemolytic Anemia in Chronic Lymphocytic Leukemia. Therapy of symptomatic autoimmune hemolytic anemia, particularly in association with chronic lymphocytic leukemia, has been substantially improved by the introduction of corticosteroids and ACTH. Not only do these hormones appear to have a favorable effect upon the autoantibody-induced process of red cell destruction but in some cases they also exert a definite antileukemic effect. Since the use of these hormones in autoimmune hemolytic anemia has been reviewed by others [*6,42,45*], only a few specific aspects of such therapy will be discussed here.

Patients experiencing sudden severe hemolysis, who are unable to develop adequate compensatory erythropoiesis, represent a medical emergency. Relatively large doses of hydrocortisone, 200 to 400 mg. in the first twelve to twenty-four hours, should be given intravenously and should be followed by relatively large oral doses of corticosteroid. For the latter purpose one of the synthetic steroids having less of the salt-retaining effect, such as prednisone in doses of 40 to 80 mg. per day, is preferred.

Prompt blood transfusion may be needed in such cases and may be life-saving. However, the serologic problem of finding a truly compatible donor may be formidable. Furthermore, transfused cells, which usually have a mean age greater than that of the patient's own circulating red cells, may be destroyed very rapidly in the presence of autoantibody. The intensity of the hemolytic process is thus frequently increased after transfusions. For these reasons it is wise to refrain from giving transfusions to patients with autoimmune hemolytic anemia, if possible, by using all other means available to support the circulatory functions. If circulatory failure

or anoxia threatens, transfusion may be mandatory. It should be recognized that the beneficial effects of transfusion are likely to be temporary and may be achieved at the cost of increased hemolysis and resultant hemoglobinemia and hemoglobinuria. In this situation, transfusion and other supportive measures may sustain the patient until corticoid hormone therapy decelerates hemolysis sufficiently to stabilize the patient's red cell mass at an adequate level.

Patients exhibiting active hemolysis without progressively severe anemia should also be treated vigorously and promptly. Steroid hormone, given orally in relatively large doses, is usually indicated. As the hemolytic process abates and the hematocrit begins to rise, the dose of steroid can be decreased slowly to amounts equivalent to about 20 mg. of prednisone daily. We are currently inclined to maintain this dose for about three to six weeks after an acute episode of hemolysis has subsided. The dose may then be decreased slowly and therapy ultimately discontinued in most cases.

Splenectomy was not considered necessary in nine of ten patients in view of the adequate response to administration of ACTH or corticosteroid. However, we would not hesitate to recommend splenectomy if hemolysis persisted despite intensive hormone therapy and if the spleen were at least moderately enlarged and the patient's general condition were considered favorable for operation. Splenectomy was performed with apparently good response in one of our patients before hormone therapy became available (Case R. C., previously reported [*46*]). Prolonged steroid hormone therapy is especially hazardous in patients with leukemia. Infection, already a problem in many patients because of poor immune and cellular defenses, may be more serious during administration of corticosteroids.

Appropriate antileukemic therapy should be administered if the patient's leukemic process is active. At times, hemolysis seems to decrease as the leukemic process comes under control. Administration of an alkylating agent may be effective occasionally but its use should be carefully considered as part of the over-all therapy of the patient, weighing the possible beneficial antileukemic effect against the almost certain effect of temporarily inhibiting red cell production to some degree.

It has been the experience of several authors, as well as our own, that steroid therapy is distinctly less effective in patients with reticuloendothelial malignancies and acquired hemolytic anemia in whom evidence of autoimmunization is not found [*42*]. In such patients the hemolytic process may be completely intractable. Splenectomy may reduce the rate of red cell destruction in some of these patients for a variable period of time but the indications for this procedure are not clear at present. Evidence of sequestration and/or destruction of red cells in a greatly enlarged spleen, demonstrated by the infusion of Cr^{51}-labelled normal red cells and episplenic radioactivity measurements, may favor splenectomy. These findings do not guarantee that the result of this operation will be useful or long-lasting. The hazard of operation must be measured against the uncertainty of outcome.

Blood Loss. At some point in the illness many patients with leukemia suffer from hemorrhage which may result in anemia if sufficient blood is lost. Little comment need be made about those situations in which blood loss is apparent and massive. However, occult bleeding, especially into the gastrointestinal tract, may also be significant. Stools should be examined frequently for the presence of blood. When found, the possibility of a specific bleeding lesion, independent of the leukemic state, should be considered and investigated. Coexistence of peptic ulcer and leukemia, or the appearance of peptic ulceration and bleeding in patients receiving corticosteroids for long periods, are among the more frequently encountered problems of this sort. Inability of the bone marrow to respond with increased erythropoiesis may result in clinically significant anemia after inconspicuous bleeding.

Epistaxis and excessive menstrual blood loss are two particularly troublesome kinds of bleeding in leukemic patients. Epistaxis is best controlled with appropriate nasal packs. Cauterization and other tissue-damaging procedures, which may result in more severe bleeding later, should be avoided. Similarly, suppression of menses, either hormonally or by radiation, may be indicated in the management of menorrhagia occurring in leukemia. Intrauterine packs should be avoided if possible.

Since leukemic patients bleed because of thrombocytopenia, transfusions of fresh whole blood, which may also provide intact platelets, should be considered in the management of this condition [*54*]. The hemostatic effect of this type of transfusion may be limited because of

the development of platelet isoantibodies in patients who have been repeatedly transfused [*55*]. The effect is at best short-lived since the life span of platelets is only four to six days. However, if a hemostatic effect can be obtained even briefly, local hemostasis may proceed to a point at which bleeding is controlled.

Diffuse capillary bleeding into many organs, as well as the skin, subcutaneous tissues and muscle, may be viewed as another type of blood loss. Although some of these red cells may find their way back to the circulation via the lymphatic system, it seems likely that they are damaged in tissue sites and have a short life span after return to the blood. The quantitative aspect of this type of blood loss is difficult to assess but at times it seems quite impressive as estimated clinically.

Anemia Related to Antileukemic Treatment. Treatment of the leukemic state itself may be responsible for some degree of anemia by inhibiting the production of red cells. All classes of antileukemic agents, except corticoid hormones, have this undesirable side effect in varying degree. Alkylating agents, including nitrogen mustard, triethylene melamine (TEM), chlorambucil, busulfan and a number of other drugs with similar action, have considerable inhibitory effect on the production of red cells when given in sufficiently large doses. The magnitude of this effect varies from drug to drug and from patient to patient. Nitrogen mustard and TEM are especially hazardous from this point of view. Radiation, external or produced by a radioisotope such as P^{32}, has a similar effect when administered to large body segments or in large doses.

Antimetabolites, such as 6-mercaptopurine, exert a less predictable inhibitory effect on erythropoiesis. Both 6-mercaptopurine and folic acid antagonists may suppress multiplication of erythroid cells, as well as leukocytes, by inhibiting nucleoprotein synthesis. After prolonged administration the folic acid antagonists may induce a true megaloblastic pattern of erythropoiesis in a few patients, and severely depress the erythropoietic rate [*4*].

Little can be done to minimize these undesirable side effects of antileukemic therapy upon red cell production. Their occurrence again emphasizes the importance of well planned therapeutic programs in patients with leukemia and of avoiding unnecessary therapy. In all instances the prospective gain of treatment should outweigh this and other therapeutic hazards, such as the production of thrombocytopenia.

Therapy with so-called "hematinic agents" is rarely beneficial in patients with anemia secondary to leukemia. Not only is such therapy ineffective, unnecessary and, frequently, expensive, but it may also be harmful at times. Preparations such as the typical "shot-gun hematinic" containing folic acid may exert an untoward effect in patients with myelocytic types of leukemia [*56*]. At best, this type of uncritically administered therapy beclouds the physician's view of the fundamental nature of the problem and of its more effective management.

Transfusion Therapy. Blood transfusion is the cornerstone of the management of anemia in the leukemic patient, except in those patients with symptomatic autoimmune hemolytic anemia. A moral issue is frequently raised as to the propriety of administering blood, always in short supply, to patients with rapidly fatal types of leukemia. Most physicians elect to give these patients transfusions if blood is available and if transfusions improve the patient's sense of well-being or add significantly to his comfort or functional capacity. We concur in such a policy, although we consider it unwise to persist with massive transfusion therapy when the disease has progressed to a point at which this procedure merely adds to the patient's discomfort and all real usefulness is lost.

Patients with chronic leukemia and severe anemia, but with the prospect of a long period of survival, constitute a more clear-cut indication for transfusion therapy. In such patients transfusion may make the difference between a comfortable life and severe invalidism. Regularly spaced transfusions should be given in an amount necessary to give the patient a reasonable functional capacity. It is seldom necessary to maintain the hematocrit above 25 per cent; indeed, large amounts of blood may be required to maintain higher levels. Regular transfusions of 1 to 2 units are preferable to permitting the patient to become severely anemic and then transfusing large volumes of blood within a short period.

SUMMARY

The pathogenetic mechanisms, clinical manifestations and treatment of anemia in the various forms of leukemia is discussed. Cases are cited to illustrate "preleukemic" hypoplasia of the bone marrow and abnormalities of the red cells

encountered in patients with leukemia and with myeloid metaplasia.

Particular reference is made to the role of hemolytic mechanisms. In some cases, notably in acute leukemia and in myeloid metaplasia, abnormalities in the shape of the red cells, i.e., poikilocytosis, spherocytosis and leptocytosis, with corresponding abnormalities in osmotic fragility, may be striking. In other cases extracorpuscular factors appear to be largely responsible for hemolysis. The most clearly identified examples of such factors are the autoantibodies encountered in chronic lymphocytic leukemia. Other extracorpuscular hemolytic agents or mechanisms are no doubt operative in leukemia but cannot be identified as yet.

Inability of the bone marrow to respond, for various reasons, to increased demands for erythropoiesis imposed by accelerated destruction of red cells and/or by blood loss is a general pathogenetic mechanism encountered frequently in leukemia of all types.

The observations cited emphasize the point that the leukemias are a group of diseases in which the cellular abnormalities are by no means confined to the leukocytes.

REFERENCES

1. Dale, J. H., Jr. Leukemia in childhood. *J. Pediat.*, 34: 421, 1949.
2. Brandberg, O. Studien über das klinische Bild der Leukosen, der sog. leukämoiden Reaktionen im Kindesalter. *Acta paediat.* (supp. 1), 30: 1, 1943.
3. Kirshbaum, J. D. and Preuss, F. S. Leukemia. A clinical and pathologic study of one hundred and twenty-three fatal cases in a series of 14,400 necropsies. *Arch. Int. Med.*, 71: 777, 1943.
4. Wintrobe, M. M. Clinical Hematology. Philadelphia, 1956. Lea & Febiger.
5. Berlin, R. Red cell survival studies in normal and leukaemic subjects. *Acta med. scandinav.* (Supp. 252), 139: 1, 1951.
6. Dameshek, W. and Gunz, F. Leukemia. New York and London, 1958. Grune & Stratton.
7. Neumann, E. Ueber myelogene Leukämie. *Berl. klin. Wchnschr.*, 15: 69, 1878.
8. Gowers, W. R. Splenic leucocythaemia. In: System of Medicine, vol. 3, p. 475. Edited by Reynolds, J. R. Philadelphia, 1880. Henry C. Lea's Son & Co.
9. Hirschfeld, H. Ueber Leukanämie. *Folia haemat.*, 3: 332, 1906.
10. Paschkis, K. Ueber atypische hämolytische Anämien. *Ztschr. f. klin. Med.*, 105: 301, 1927.
11. Whipple, G. H. and Robscheit-Robbins, F. S. Hemoglobin production factors in the human liver. III. Anemias-primary, aplastic, and secondary-leukemias. *J. Exper. Med.*, 57: 671, 1933.
12. Huff, R. L., Hennessy, T. G., Austin, R. E., Garcia, R. F., Roberts, B. M. and Lawrence, J. H. Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders. *J. Clin. Invest.*, 29: 1041, 1950.
13. Ross, J. F., Crockett, C. L., Jr. and Emerson, C. P. The mechanism of anemia in leukemia and lymphoma. (Abstract.) *J. Clin. Invest.*, 30: 668, 1951.
14. Berlin, N. I., Lawrence, J. H. and Lee, H. C. The life span of the red blood cell in chronic leukemia and polycythemia. *Science*, 114: 385, 1951.
15. Berlin, N. I., Lawrence, J. H. and Lee, H. C. The pathogenesis of the anemia of chronic leukemia. Measurement of the life span of the red blood cell with glycine-2-C^{14}. *J. Lab. & Clin. Med.*, 44: 860, 1954.
16. Weinstein, I. M. and LeRoy, G. V. Radioactive sodium chromate for the study of survival of red blood cells. II. The rate of hemolysis in certain hematologic disorders. *J. Lab. & Clin. Med.*, 42: 368, 1953.
17. Jaffé, R. H. Erythropoiesis in leukemia. *Folia haemat.*, 49: 51, 1933.
18. Jaffé, R. H. The nature of the anemia in acute leukemia. *Arch. Path.*, 20: 725, 1935.
19. Beyers, M. R., Meyer, L. M., Lowenthal, M., Oehrig, R. J. and Sawitsky, A. Hypocellular marrow in acute leukemia. *Arch. Int. Med.*, 88: 803, 1951.
20. Adams, E. B. Aplastic anemia: review of twenty-seven cases. *Lancet*, 1: 657, 1951.
21. Block, M., Jacobson, L. O. and Bethard, W. F. Preleukemic acute human leukemia. *J. A. M. A.*, 152: 1018, 1953.
22. Bernard, J. and Boiron, M. Les anémies preleucoblastiques de la leucose aigue. *Sang*, 25: 797, 1954.
23. Propp, S. Subleukemic leukemia, a common type of leukemia, with emphasis on its hypoplastic form. In: Proceedings of the Sixth International Congress of the International Society of Hematology. New York, 1958. Grune & Stratton.
24. Reisner, E. H., Jr. The nature and significance of megaloblastic blood formation. *Blood*, 13: 313, 1958.
25. Baldini, M., Fudenberg, H. H., Fukutake, K. and Dameshek, W. The anemia of the DiGuglielmo syndrome. *Blood*, 14: 334, 1959.
26. Maloney, W. C. Leukemia and exposure to x-ray: a report of 6 cases. *Blood*, 14: 1137, 1959.
27. Court-Brown, W. M. and Doll, R. Leukemia and aplastic anemia in patients irradiated for ankylosing spondylitis. In: Medical Research Council Special Series, No. 295, Her Majesty's Stationery Office, 1957.
28. Brodell, H. and Swisher, S. N. Studies of leukocyte alkaline phosphatase by a clinically applicable histochemical method. (Abstract.) *Clin. Res. Proc.*, 2: 58, 1954.
29. Meacham, G. C. and Weisberger, A. S. Early atypical manifestations of leukemia. *Ann. Int. Med.*, 41: 780, 1954.
30. Williams, M. J. Myeloblastic leukemia preceded by prolonged hematologic disorder. *Blood*, 10: 502, 1955.
31. Vilter, R. W., Jarrold, T., Will, J. J., Mueller, J. F., Friedman, B. I. and Hawkins, V. R. Refrac-

tory anemia with hyperplastic bone marrow. *Blood*, 15: 1, 1960.

32. Wintrobe, M. M. and Mitchell, D. M. Atypical manifestations of leukemia. *Quart. J. Med.*, 9: 67, 1940.
33. Young, L. E., Platzer, R. F. and Lawrence, J. S. Unusual anemia associated with chronic myeloid leukemia. *Acta haemat.*, 5: 345, 1951.
34. Nathan, D. G. and Berlin, N. I. Studies of the rate of production and life span of erythrocytes in acute leukemia. *Blood*, 14: 935, 1959.
35. London, I. M., Shemin, D., West, R. and Rittenberg, D. Heme synthesis and red blood cell dynamics in normal humans and in subjects with polycythemia vera, sickle-cell anemia and pernicious anemia. *J. Biol. Chem.*, 179: 463, 1949.
36. Wetherley-Mein, G., Epstein, I. S., Foster, W. D. and Grimes, A. J. Mechanisms of anaemia in leukaemia. *Brit. J. Haemat.*, 4: 281, 1958.
37. Dacie, J. V. and White, J. C. Erythropoiesis with particular reference to its study by biopsy of human marrow: a review. *J. Clin. Path.*, 2: 1, 1949.
38. Cartwright, G. E. and Wintrobe, M. M. The anemia of infection. xvii. A review. In: Advances in Internal Medicine, vol. 5, p. 165. Chicago, 1952. Year Book Publishers, Inc.
39. Young, L. E., Izzo, M. J., Altman, K. I. and Swisher, S. N. Studies on spontaneous in vitro autohemolysis in hemolytic disorders. *Blood*, 11: 977, 1956.
40. Selwyn, J. G. and Dacie, J. V. Autohemolysis and other changes resulting from the incubation in vitro of red cells from patients with congenital hemolytic anemia. *Blood*, 9: 414, 1954.
41. Crosby, W. H. and Benjamin, N. An abnormal hemolytic system associated with leukemia and other disseminated malignant diseases. *Blood*, 12: 701, 1957.
42. Wasserman, L. R., Stats, D., Schwartz, L. and Fudenberg, H. Symptomatic and hemopathic hemolytic anemia. *Am. J. Med.*, 18: 961, 1955.
43. Dacie, J. V. The Haemolytic Anaemias. New York, 1954. Grune & Stratton.
44. Dacie, J. V. The auto-immune haemolytic anaemias. *Am. J. Med.*, 18: 810, 1955.
45. Rosenthal, M. C., Pisciotta, A. V., Komninos, Z. D., Goldenberg, H. and Dameshek, W. The auto-immune hemolytic anemia of malignant lymphocytic disease. *Blood*, 10: 197, 1955.
46. Young, L. E. and Miller, G. The long-term picture in autoimmune hemolytic disease. *Tr. A. Am. Physicians*, 66: 190, 1953.
47. Swisher, S. N. Autoimmune hemolytic disease: some experiences and some unsolved problems. In: Mechanisms of Hypersensitivity. Boston, 1959. Little, Brown & Co.
48. Miller, G., Shumway, C. N. and Young, L. E. Autoimmune hemolytic anemias. *Pediat. Clin. North America*, February, 1957.
49. Dausset, J. and Colombaric, J. The serology and the prognosis of 128 cases of autoimmune hemolytic anemia. *Blood*, 14: 1280, 1959.
50. Goudsmit, R. and Krijnen, H. W. Increased destruction of "compatible" red cells. *Vox Sang.*, 2: 357, 1957.
51. Jandl, J. H. and Greenberg, M. S. The selective destruction of transfused "compatible" normal red cells in two patients with splenomegaly. *J. Lab. & Clin. Med.*, 49: 233, 1957.
52. Mollison, P. L. Blood group antibodies and red cell destruction. ii. *Brit. M. J.*, 1: 1123, 1959.
53. Swisher, S. N. Studies of the mechanisms of erythrocyte destruction initiated by antibodies. *Tr. A. Am. Physicians*, 67: 124, 1954.
54. Gardner, F. H., Howell, D. and Hirsch, E. O. Platelet transfusions utilizing plastic equipment. *J. Lab. & Clin. Med.*, 43: 196, 1954.
55. Gardner, F. H., Aas, K. A., Cohen, P. and Pringle, J. C. Investigations of the mechanisms of thrombocytopenia. *Clin. Res.*, 6: 199, 1958.
56. Farber, S. The chemotherapeutic studies of tumors, including leukemia, in children. *Am. J. Dis. Child.*, 79: 961, 1950.

Clinical Manifestations of Leukemia*

Recent Contributions

BARRY FIRKIN, M.B.B.S.† *and* CARL V. MOORE, M.D.
St. Louis, Missouri

INCLUSION of a paper on the clinical manifestations of leukemia in a symposium designed to emphasize the current investigative work on that disease places special demands on the clinical discussion. An orderly description of signs and symptoms and of involvement of the various organ systems would require repetition of material readily available in standard textbooks or monographs, and would seem out of place. We have decided, therefore, to concentrate on a review of relatively new information about the occurrence, clinical course and manifestations of leukemia.

CLASSIFICATION AND OCCURRENCE

The simple classification of leukemia into acute and chronic forms of myelocytic, lymphocytic and monocytic types is the most useful. The term "stem cell" leukemia is reserved for those cases of the acute disease in which the blast forms are so primitive that the observer is unable to classify them as precursors of one of the definitive cell types. When the total white blood cell count is normal or low, "subleukemic" is a better adjective than "aleukemic." Particularly troublesome problems related to classification are the following:

(1) Precise definitions separating acute and chronic forms of leukemia have become more difficult since therapy with the newer chemotherapeutic drugs has prolonged the life of patients with acute leukemia, particularly in children, to as long as several years. Even when antileukemic therapy is not employed, patients with the acute disease may survive three to six months or longer while a small percentage of those with chronic leukemia die within twelve months of the onset of symptoms [*1*]. Until the etiology is known and shown to be different, or until specific cytochemical differentiation becomes possible, the differentiation of acute leukemia from chronic leukemia is best made on the degree of immaturity—the number of blast forms—in the peripheral blood and bone marrow. It is evident that on this basis there will be cases difficult or impossible to classify with precision, a difficulty only partially resolved by use of the term "subacute."

(2) Monocytic leukemia is regarded by some hematologists, particularly those who use supravital as well as the Romanowsky staining technics, as constituting one type [*2*], while others differentiate a histiocytic (Schilling) and a myelomonocytic (Naegeli) variety, and classify the latter with myelocytic leukemia [*3*]. As a result, the percentage of cases diagnosed as monocytic leukemia will vary in different laboratories from 2 to 4 per cent to as high as 17 per cent. At Washington University, for instance, 288 of a total of 1,669 patients with all types of leukemia were regarded as monocytic (17 per cent); probably over half of these would have been classified by other workers as acute myelocytic. Chronic forms of monocytic leukemia are rare.

(3) The term leukosarcoma is used in two different ways to designate a variety of acute lymphocytic leukemia: (a) as the terminal leukemic phase of some patients with lymphosarcoma; and (b) as a form of acute lymphocytic leukemia in which the abnormal cells in the blood and bone marrow are thought to have the morphologic characteristics of lymphosarcoma cells even though no lymphosarcomatous phase of the disease can be recognized. Confusion can be obviated if the latter group of cases is regarded simply as acute lymphocytic leukemia, at least until the etiology of acute leukemia is understood and we know whether one or several etiologic factors are involved.

* From the Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.
† Lederle International Fellow and U. S. Public Health Service Trainee.

TABLE 1

COMPARISON OF THE INCIDENCE OF ACUTE LEUKEMIAS AND OF CHRONIC LYMPHOCYTIC LEUKEMIA IN ASIA, THE UNITED STATES AND GREAT BRITAIN

Author	Country	Total Cases	Per cent Acute	Chronic Leukemia*		
				CLL No.	Per cent	Ratio CML/CLL
Wakisaka, G. [6]	Japan	1,959	78.9	40	2.0	10.7†
Shiraishi, T. [7]	Japan	781	82.4	15	1.9	9.3†
Ota, K. [8]	Japan	618	76.7	6	1.0	23.0
Ting, N., Hwang, T. [9]	Taiwan	61	72.1	3	4.9	4.7
Chatterjea, J. B., Ghosh, S., Ray, R. N., Banerjee, D. K. [10]	India	178	55.6	8	4.4	8.9
Grieg, H. W. B. [11]	S. African Bantus	47	30.0	11	23.5	2.0
	S. African Whites	86	53.5	27	31.4	0.5
Wintrobe, M. M. [12]	U.S.A. and Great Britain	2,424	40.1	747	30.9	0.9
Washington University	U.S.A.	1,669	36.7	627	37.5	0.7

* CLL = chronic lymphocytic leukemia. CML/CLL = chronic myelocytic leukemia/chronic lymphocytic leukemia.

† Calculated from cases derived from hospital records only. The data tabulated by Wintrobe, and the figures from Washington University are cited as representative examples; for a more complete tabulation of incidence, reference should be made to the monograph by Dameshek and Gunz [3]. The data from our laboratory are distorted by the fact that children are usually seen only by the hematology service of the Department of Pediatrics; our incidence of acute lymphocytic leukemia, therefore, is lower than it would be if all children seen at this medical center were included in the compilation.

(4) Uncertainty exists as to whether multiple myeloma should be classified, as in the past, as a separate disease or as plasmocytic leukemia, usually subleukemic.

(5) Unusual forms of leukemia are encountered which are classified with difficulty: the Di Guglielmo syndrome with simultaneous proliferation of myeloblastic and erythroblastic cells, mast cell leukemia and leukemic reticuloendotheliosis. Basophilic and eosinophilic leukemias are variants of the myelocytic type.

The difficulties represented by the examples cited are a reflection of the fact that when the etiologic factors responsible for the proliferation of abnormal cells are unknown, our knowledge about cellular physiology and biochemistry is inadequate to support the information available from morphologic studies alone. Fortunately, most cases of leukemia can be fitted adequately into the simple classification first given, and any uncertainties have little clinical implication.

Evidence that the incidence of leukemia is increasing at a rapid rate continues to accumulate, but uncertainty still exists whether the rise is real or a reflection of more accurate diagnosis [3,4]. The death rate from leukemia in the United States for 1956 was estimated to be 6.82 per 100,000 population [5]. Males are more frequently affected, particularly with chronic lymphocytic leukemia, than females (7.9 versus 5.7 deaths per 100,000). Acute lymphocytic leukemia continues to be the predominant form in childhood, myelocytic leukemia has its greatest incidence during middle life, and chronic lymphocytic leukemia in the older age groups. The two most significant and interesting new data about occurrence of leukemia, however, are these:

(1) The increase in incidence of leukemia among older people is significantly greater than for younger groups, seems to be continuing, and is predominantly of the chronic lymphocytic type. Since chronic lymphocytic leukemia is only rarely encountered in patients in whom leukemia develops after exposure to large amounts of ionizing radiation, it becomes difficult to attribute the apparent increased incidence of the last several decades among the population at large to greater exposure to radiation.

(2) In Japan, India, and possibly all of Asia, chronic lymphocytic leukemia is much less common than in North America and Europe (Table 1), the reported ratio of chronic myelocytic leukemia to chronic lymphocytic leukemia

varying from 5 to about 20 to one. In addition, the incidence of acute leukemia in Japan is considerably higher than in the western countries. The reasons for these dramatic differences are still obscure. Information from Africa is too meager to permit any conclusions. Somewhat more minor differences in incidence of leukemia among other ethnic groups have been described in the United States: a smaller over-all incidence among American Negroes [*3*] and a somewhat higher rate among Jews [*13*]. Differences in diagnostic methods and medical services available to various population groups make it unwise to place too much attention on minor variations, but the low rate of chronic lymphocytic leukemia in Asia is most impressive, and is too great to be explained on the basis of differences in life expectancy [*6*].

Differences in the age and ethnic distribution of the various types of leukemia strongly suggest that more than one etiologic factor must be responsible for the disease.

PRELEUKEMIC LEUKEMIA

During recent years an increasing number of patients have been recognized in whom leukemia develops after an hematologic illness of varying duration. While isolated reports of such patients had been published, not until Block and his associates [*14*] described twelve cases did the concept of a preleukemic state develop. These patients may appear to have refractory anemia, neutropenia, thrombocytopenia or a combination of the three. Less commonly, the presenting features are those of a Coombs-negative hemolytic anemia [*15*] or rarely a megaloblastic anemia [*16*]. The bone marrow is rarely truly aplastic; hyperplasia of erythroid elements with hypoplasia or hyperplasia of the granulocytic series is frequently found [*14*]; and no evidence of leukemia can be recognized. In the peripheral blood the number of reticulocytes may be increased and a few normoblasts may be seen. The serum bilirubin may be elevated and the excretion of fecal urobilinogen may be greater than normal. There is no significant enlargement of the lymph nodes; the liver and spleen are usually not palpable. Purpura is sometimes present and lesions of the mucous membranes tend to heal poorly [*14*]. The illness pursues a benign course for months to a few years before the catastrophic change to leukemia occurs. The recognized onset of leukemia is sometimes heralded by a peripheral monocytosis plus increased hyperplasia of marrow and immaturity of granulocytic cells; it is often accompanied by enlargement of the lymph nodes, liver and spleen. Acute myelocytic (or "stem cell") leukemia is the most common form, but monocytic leukemia or changes resembling chronic myelocytic leukemia have been observed [*14,15*]. The course of the terminal episode is usually precipitous and characterized by failure to respond adequately to any known form of therapy. At present, the preleukemic state cannot be distinguished from aplastic anemia since the changes in bone marrow may be similar, and leukemia has developed in patients whose bone marrow was initially extremely hypocellular [*17*].

The important question, for which no answer is available, is this: Do these patients have leukemia in a form unrecognizable with current diagnostic methods from the onset of their illness, or does the preexisting hematologic abnormality predispose them to the subsequent development of leukemia? In this regard it should be remembered that acute leukemia may occur terminally in patients with polycythemia and myelofibrosis. While the marrow is more frequently cellular than aplastic during the preleukemic period, it is at least interesting that an impressive amount of evidence links hypoplasia of the marrow and leukemia. Radiation and possibly benzol are capable of inducing either aplasia or leukemia. Although the cause and effect relationship has by no means been established, leukemia following the aplasia supposedly produced by the administration of chloramphenicol and sulfonamides has been described [*18*]. Studies of families with hereditary hypoplastic anemia (Fanconi's syndrome) have shown an increased incidence of luekemia [*19*]. In one reported case of acute leukemia, four separate spontaneous remissions were preceded by agranulocytosis and aplasia of the marrow [*20*]. The claim has been made that nests of leukemic cells may be found at autopsy in the marrow of some patients with idiopathic aplastic anemia [*21*]. More pertinent perhaps is the observation that changes in the peripheral blood (leukocytosis, relative lymphopenia, presence of neutrophilic metamyelocytes and myelocytes in small numbers, increase in basophils and in the platelet level) could be detected in a group of patients exposed to atomic radiation in Hiroshima in whom chronic myelocytic leukemia developed later; similar changes were not observed when the subsequent leukemia was acute [*22*]. The lag

period between exposure to radiation and the appearance of leukemia, plus animal studies showing alterations in the thymic nucleic acid pattern of radiated animals (increase in RNA) before the development of leukemia [*23*], are consistent with the concept of a preleukemic state. None of these observations, however, provides a satisfactory answer to the question asked at the beginning of this paragraph.

Why leukemia occurring as a terminal event in the course of refractory or hemolytic anemia was not recognized with regularity until the last decade is a mystery. The changes are so dramatic that no special diagnostic procedures are required for recognition. They may have escaped detection, but the following report of acute myelocytic leukemia occurring in a patient who had been treated over a long period for refractory anemia illustrates how difficult it would be to ignore the change.

R. C., a Jewish business executive sixty-nine years of age, was found to have a moderate macrocytic anemia in 1945 when he was admitted to the Barnes Hospital for treatment of erysipelas; his physician attributed the anemia to the infection. The red blood cell count was 3,130,000 per cu. mm., the hemoglobin 12.2 gm. per cent, and the white blood cell count was normal. The patient was asymptomatic until May 1951 when he noticed pallor and increasing tiredness. Physical examination at that time was within normal limits. The blood count was as follows: red blood cells 2,580,000 per cu. mm., hemoglobin 10.9 gm. per cent, reticulocytes 1.8 per cent, white blood cells 7,650 per cu. mm., differential and platelet count normal. Material aspirated from the sternum contained many clumps of cellular marrow; the erythroid elements were hyperplastic, with an increase in the number of polychromatophilic normoblasts; granulocytic and megakaryocytic elements appeared normal. Hepatic function tests, serum non-protein nitrogen, protein-bound iodine and basal metabolic rate were all normal. No improvement followed the administration of folic acid, folinic acid and vitamin B_{12}.

The peripheral blood values and bone marrow cytology were essentially unchanged in August 1951. The result of the Coombs' test was negative. The serum iron on two occasions was 368 and 354 μg. per 100 ml. with complete saturation of the iron-binding protein. Fecal urobilinogen excretion was 410 mg. per day with a hemolytic index of 62. The life span of transfused erythrocytes (Ashby technic) was eighty to ninety days. Only 27 per cent of a tracer dose of radioiron was utilized for hemoglobin formation and appeared in the circulating erythrocytes. The anemia, therefore, was interpreted as being primarily due to decreased erythropoiesis together with a mild hemolytic component; a diagnosis of refractory anemia or bone marrow failure was made.

During the next four years the patient remained fairly comfortable except for weakness when his hemoglobin fell below 8 gm. per cent; after transfusions his feeling of relative well-being returned and he continued to direct his business. Altogether, fifty-five transfusions were given during these four years. There was no significant change in the leukocyte count or differential, in the reticulocyte and platelet levels, or in the physical examination except for bronzing of his skin. Except for brief periods when he left the city for vacations, he was seen at intervals of one to three weeks.

Early in July 1955, his weakness became more profound, he lost his appetitie, and fever developed. An increase in monocytes was observed in the peripheral blood, together with a few myelocytes; shortly thereafter blast forms appeared and the total white blood cell count rose to 18,000 per cu. mm. His spleen was felt for the first time, 5 cm. below the costal margin. Many immature granulocytes and blast forms were found in the aspirated sternal marrow and erythroid elements were now decreased in number. From this time until he died on April 27, 1956, his course was that of acute myelocytic leukemia. His spleen and liver became so large as eventually to be palpated 18 and 11 cm., respectively, below the left and right costal margins. He had one episode of pneumonia, several attacks of pain in the left upper quadrant which were interpreted as probably caused by splenic infarcts, and a painful perirectal abscess developed. The white cell count was frequently above 100,000 per cu. mm. and was found to be 334,500 per cu. mm. on April 10; on that day the per cent of the various leukocytes was 10 band forms, 2 metamyelocytes, 3 myelocytes, 6 promyelocytes, 70 myeloblasts, 1 lymphocyte, and 8 monocytes. The platelets decreased to low levels and reticulocytes were rarely found. Therapy with 6-mercaptopurine, prednisone, Myeleran,® and antibiotics when indicated failed to cause any improvement. During the last few weeks of life cardiac failure developed with left pleural effusion. Transfusions had to be given at least twice a week in order to maintain the hemoglobin level above 7 gm. per cent.

At autopsy severe bronchopneumonia was found in both lungs and multiple, organizing pulmonary thrombi were present bilaterally; these changes were thought to be the immediate cause of death. The spleen weighed 1,250 gm. and the liver 3,650 gm. Leukemic infiltrates were present in the spleen, lymph nodes, liver, left renal pelvis and small bowel. There was marked hemosiderosis of the liver, kidneys, adrenals, prostate, lymph nodes, bone marrow and pancreas. The bone marrow was hyperplastic and consisted of an almost solid mass of leukemic blast forms, consistent with a diagnosis of acute myelocytic leukemia.

Comment: The rather abrupt change in the hematologic and clinical manifestations to those of acute leukemia is characteristic of the chain of events described by Block and his associates [*14*], but the long preleukemic refractory anemia period (four or possibly even ten years) is much longer than usual. It is difficult to believe that the disease was really unrecognized leukemia during the whole of that time.

CLINICAL MANIFESTATIONS

As patients with leukemia, particularly of the acute variety, have tended to live longer under the influence of modern therapy and as new methods of study have become available, new clinical signs have been recognized or more adequate explanation of certain manifestations of the disease has been provided. This more recent information will be reviewed; reference should be made to standard textbooks for description of the clinical course and involvement of the various organ systems.

Fever and Infection. The fever frequently associated with any form of leukemia has been attributed to the general hypermetabolic state of the patients, to the tissue destruction-like effect produced by the turnover of large numbers of white blood cells, and/or to infection. The first two of these possible mechanisms fail to explain the absence of fever in some patients and the lack of correlation between the degree of fever and the leukocyte count, the severity of anemia and the presence or absence of hemorrhage. For these reasons, and because better diagnostic methods are now available, the importance of infection in the causation of fever in patients with leukemia needs to be reevaluated. Such a study has been made on thirty-six consecutive patients with acute leukemia by Silver and his associates [*24*], using comprehensive bacterial, fungal and viral technics. Of the 2,614 patient days of observation in the hospital, fever was present nearly half the time. In only two patients was the temperature consistently normal. Fifty-nine of the ninety-two febrile episodes recorded appeared to be related to infection; it was proved in forty-four instances and presumptive in fifteen. The most common clinical illnesses associated with proved infections were pharyngitis, pyelonephritis and septicemia; multiple sites of infection often occurred. The most frequently encountered organisms were Escherichia coli, Micrococcus pyogenes var. aureus (coagulase-positive), and Pseudomonas aeruginosa; there was an almost equal distribution between gram-positive and gram-negative bacteria; on occasion relatively avirulent organisms were responsible for serious clinical infections. The majority of the presumptive infections were related to infections of the respiratory tract. In the remaining thirty-three febrile episodes, bacterial, fungal or viral infection could not be incriminated and the fever was of undetermined origin. A therapeutic trial of antibiotic administration was made: fourteen patients became afebrile when given penicillin and streptomycin or Terramycin®; five children treated both with antibiotics and corticosteroids became afebrile within four days; in fourteen patients the fever persisted after antibiotic therapy. Ten of these last fourteen patients were children. If the patients who responded to the administration of antibiotics, even though no infecting organism was identified, are regarded as having infection-induced fevers, then only a small percentage of the febrile episodes remained unexplained. The failure to identify any viral infections is of interest, particularly since viruses were isolated from non-leukemic patients by this laboratory during the period of study.

Disseminated secondary fungal infections in patients with both acute and chronic leukemia have become a troublesome problem in recent years. Candidiasis, cryptococcosis, histoplasmosis and mucormycosis account for the increased incidence of fungal infections at Washington University [*25*]. In the study by Silver et al. [*24*], candida (but no other fungi) were isolated from eighteen of thirty-six patients; signs of infection were present in only eleven: confined to the pharynx in eight, moniliasis of the pharynx, skin and bladder in two, and monilial enteritis in one. The suggestion has been made that the more frequent occurrence of complicating disseminated secondary fungal infections may be related to the use of antibiotics, adrenal steroids and antileukemic chemotherapeutic compounds [*25*]. Similar considerations may be partly responsible for the experience at the Clinical Center of the National Institutes of Health where, during the period 1954 through 1957, Ps. aeruginosa was the second most frequent cause of septicemia and the most frequent bacterial cause of death on the leukemia service [*26*]. Another unusual cause of septicemia in four leukemic patients being treated with antimetabolites or corticosteroids was clostridial organisms; gas gangrene of the bowel was found and lesions pro-

duced by extensive leukemic infiltrate of the bowel were thought to be the primary sites of infection [*27*].

Abnormalities of Plasma Proteins in Leukemia, and Their Clinical Significance. Acute leukemia: With the exception of acute plasmocytic leukemia, striking alterations in the electrophoretic pattern of serum proteins are unusual in acute leukemia [*28*]. The not infrequent slight and apparently non-specific changes found may be related to infection, chronic debilitation or hepatic damage secondary to the disease process [*29*] or to some therapeutic agent [*30*]. The hypoalbuminemia and hyperglobulinemia occasionally observed may be corrected by antileukemic therapy [*31*].

Chronic myelocytic leukemia: Serums from patients with chronic myelocytic leukemia may also exhibit minor disturbances and rarely may have a homologous globulin component associated with abnormal plasma cells in the marrow [*32*]. By far the most constant anomaly, however, is a great increase in the binding capacity of the alpha globulin for vitamin B_{12} [*33*]. Whether the change is caused by an increased concentration of the vitamin B_{12} binding protein [*34*] or by a qualitative abnormality [*33*] is unknown. The chance detection of this phenomenon emphasizes the relative crudeness of current technics for protein analysis and suggests that only the grossest defects in protein metabolism are now detected.

Chronic lymphocytic leukemia: In contrast to the other leukemias, chronic lymphocytic leukemia is often associated with readily observable alterations in the serum proteins: hypogammaglobulinemia has been reported in 30 to 40 per cent of unselected patients [*28,35*]; a diffuse increase in gamma globulin may be found, and infrequently paraproteins may be present. The latter may be similar to myeloma proteins in electrophoretic characteristics and are seen as an abnormally migrating homogeneous peak [*36*]; they may have the physical properties of a macroglobulin [*37,38*] or cryoglobulin [*39–41*].

The pathogenesis of the protein disturbances in chronic lymphocytic leukemia is unknown. Hypogammaglobulinemia may be related to the degree of reticuloendothelial system involvement or to inability of the abnormal leukemic lymphocyte to participate in normal protein synthesis. Either one or both factors might cause diminished production of gamma globulin and depressed antibody synthesis [*42*]. Despite the latter, Coombs-positive acquired hemolytic anemia may develop in patients with chronic lymphocytic leukemia and hypogammaglobulinemia. Significant hypogammaglobulinemia may be present when the patient is first discovered to have chronic lymphocytic leukemia, but its incidence increases with the duration of the disease [*43,44*]. Infections are more frequent and more severe in these patients and the hypogammaglobulinemia usually shows no change with treatment despite hematological improvement. Indeed, the gamma globulin level often becomes progressively lower [*43*] and infective episodes may be incapacitating unless intramuscular injections of pooled gamma globulin are given. Most commonly the infections consist of recurrent bacterial pneumonias, often pneumococcal. We have seen pneumococcal meningitis in several patients. Whether or not the administration of gamma globulin has therapeutic value after the onset of an infection is uncertain. Because of poor production of antibodies [*42*], patients with chronic lymphocytic leukemia and hypogammaglobulinemia to whom adrenal steroids are given for any reason should receive regular injections of gamma globulin.

Diseases associated with paraproteinemia, multiple myeloma, macroglobulinemia and essential cryoglobulinemia, are characterized by proliferation or abnormalities in lymphocytes or plasma cells; the rare occurrence of paraproteins in the plasma of patients with chronic lymphocytic leukemia, therefore, is not too surprising. The relationship between macroglobulinemia and chronic lymphocytic leukemia is still not understood. Mackay [*38*] suggests that macroglobulinemia represents a "clonal phenomenon," and that the immunological and chemical differences exhibited by macroglobulins may result from the particular genotype of the cell which has undergone somatic mutation. Should the mutant retain its ability to synthesize protein, the various paraproteins including autoantibodies may result. Loss of the mutant's ability to synthesize protein would produce hypogammaglobulinemia provided the abnormal cell had replaced normal lymphocytes and plasma cells. The adoption of more malignant properties by the mutant would result in leukemia [*38*].

The paraproteins may be associated with Raynaud's phenomenon [*39*], hypersensitivity to cold resulting in urticarial wheals, purpuric

eruptions and ulcerations of the leg. Visual symptoms and sometimes a bleeding tendency may occur [*38*]. The infrequency of the paraproteinemia with chronic lymphocytic leukemia is indicated by the report that only six cases of essential cryoglobulinemia could be found in the world literature [*45*], and in one of these the diagnosis could be questioned [*46*].

The following case report is illustrative of many of the clinical problems encountered in patients with chronic lymphocytic leukemia and hypogammaglobulinemia. After the institution of regular injections of gamma globulin, the previous repeated infections of the upper respiratory tract became less troublesome.

M. P., a white man forty-eight years of age, was discovered to have chronic lymphocytic leukemia when a routine blood count was made in 1951; he was asymptomatic. In May 1953 he was admitted to the hospital with the symptoms and signs of pneumonia of the lower lobe of the left lung. Peripheral lymph nodes were 2 to 3 cm. in diameter, the liver was felt 7 cm. below the right costal margin, and the spleen was palpated 25 cm. below the left costal margin. The red cell count was 2,740,000 per cu. mm., hemoglobin 9.5 gm. per cent, white count 48,000 per cu. mm., differential 93 lymphocytes and 7 segmented neutrophils. Result of a Coombs' test was negative. Despite administration of penicillin, streptomycin and Terramycin, his progress was unsatisfactory and on the twelfth hospital day pericarditis developed. He improved following x-ray therapy to the spleen, pericardium and mediastinum. He received eleven 500 ml. transfusions of blood during this admission. One week after he had returned home the lymph nodes had not decreased in size but the liver and spleen extended only 4 and 10 cm., respectively, below the costal margins. The red blood cell count was now 3,780,000 per cu. mm., hemoglobin 11.5 gm. per cent and the white blood cell count 9,800 per cu. mm. Further x-ray therapy to the lymph nodes, spleen and mediastinum was given during January, June and November 1954. In August 1954 he was incapacitated by severe herpes zoster involving the left sciatic nerve, and subsequently postherpetic neuralgia developed. During this period he had frequent infections of the upper respiratory tract and complained of a cough. In February 1955, a marked decrease in gamma globulin was discovered. By June 1956, the cough had become chronic, and small amounts of sputum were produced each day. Administration of Chlorambucil® was begun.

In July, he had a severe episode of bronchitis which finally responded to Achromycin® therapy but in October 1956 another episode of bronchitis rapidly progressed to bilateral bronchopneumonia despite the administration of Achromycin. There was no significant change in peripheral lymph nodes or in the degree of hepatosplenomegaly; the white blood cell count was 14,500 per cu. mm. with 82 per cent lymphocytes. The serum electrophorectic pattern was characterized by an almost complete absence of gamma globulins. Prophylactic intramuscular injections of 10 ml. of pooled gamma globulin were given weekly; therapy with Chlorambucil was continued. The infections of the upper respiratory tract diminished greatly in severity but not in frequency; his requirements for red cell transfusions increased to 1 or 2 units of packed cells per week. Survival of his Cr^{51} tagged erythrocytes was greatly shortened (half-life of eight days); the reaction to the Coombs' test was negative. Because he failed to respond to the administration of cortisone, splenectomy was performed on May 10, 1956.

The patient was well for six weeks following discharge, still receiving his weekly gamma globulin but not requiring blood transfusions. Then about eight hours after a regular injection of gamma globulin he noted pain and swelling in his knees, and pain in the right shoulder and ankle. He was readmitted to the hosptial on July 11, 1956, chronically ill, with a temperature of 37.8°c. Generalized 0.5 to 1 cm. lymph nodes were palpated and the liver extended 10 cm. below the costal margin. Both knees were swollen and hot; the patellae ballotted readily. The red blood cell count was 2,300,000 per cu. mm., hemoglobin 9.3 gm. per cent, reticulocytes 1.6 per cent, platelets 73,000 per cu. mm., white blood cell count 171,000 per cu. mm. with 2 per cent segmented forms and 98 per cent lymphocytes. Roentgenograms were non-contributory except for the evidence of swelling of the soft tissue about the knees. Fourteen ml. of viscous, cloudy yellow fluid was aspirated from the left knee. Examination of the synovial fluid showed numerous segmented neutrophils, a few lymphocytes, but no crystals or bacteria. Cultures for tubercle bacilli were negative. Fifty milligrams of hydrocortisone were instilled into the synovial space. Blood cultures were negative. The patient became progressively weaker; on July 13 the injections of gamma globulin were suspended since it was thought that he might have developed a hypersensitivity reaction [*47*]. A few days later pharyngitis developed, he had difficulty in clearing sputum from his throat, and he had watery diarrhea. His respirations became labored, suction was required to remove secretions from the air passages, and death occurred on July 22.

Pathologic examination was limited to biopsy of organs. The changes of chronic lymphocytic leukemia were found in bone marrow, liver and lymph nodes from all areas. There was bronchopneumonia in both lungs with abscess formation.

Comment: No further hospital admissions for infection were necessary after the administration of gamma globulin was begun. Dr. Hugh

Chaplin demonstrated that the patient (blood group A) was unable to produce detectable isoagglutinins in response to a challenge with incompatible red blood cells (blood group B) [*48*] so there was objective evidence of defective antibody production. The occurrence of severe herpes zoster is of interest because of the suggestion that the incidence of this viral infection may be higher in patients with chronic lymphocytic leukemia and concomitant hypogammaglobulinemia [*43*]. The manifestations of the joints were similar to those described by Janeway et al. [*47*] in boys with congenital agammaglobulinemia after prolonged gamma globulin therapy, and may be an expression of a serum sickness-like hypersensitivity reaction. Hemolytic anemia is common in chronic lymphocytic anemia but its pathogenesis is particularly difficult to understand when hypogammaglobulinemia is also present; this association in both Coombs-positive and -negative form has been described before, and Coombs-negative acquired hemolytic anemia has been recognized in primary acquired agammaglobulinemia.

Neurologic Manifestations. Neurologic complications have been recognized in both acute and chronic leukemia for many years and have been described in detail; they include cerebral hemorrhage, signs of increased intracranial pressure, focal involvement causing cranial nerve palsies, Jacksonian epilepsy, and occasionally diabetes insipidus, leukemic meningitis, involvement of the spinal meninges or spinal nerve roots, and peripheral neuritis. During the past few years, however, among patients with acute forms of the disease, leukemic meningitis seems to have become more frequent, and the pathogenesis of intracranial hemorrhage has in part been clarified.

Leukemic meningitis occurs more frequently in acute lymphocytic leukemia than in myelocytic leukemia; it is most common in children, particularly among males. In one recently reported series of 150 consecutively studied patients, leukemic meningitis was observed in twenty-five [*49*]. The meningeal infiltration may constitute the first clinical evidence of leukemia; it may appear at any time during the course of the disease; or it may herald a relapse in treated patients. There has been considerable interest in the observation that leukemic meningitis may develop in patients, particularly children, while under treatment with antimetabolites (amethopterin, 6-mercaptopurine) even though a satisfactory hematologic remission has been obtained. The peripheral blood and bone marrow remission, furthermore, may persist for weeks after the appearance of the meningitis. The two most likely explanations for the apparently greater recent incidence of leukemic meningitis are: (1) the longer life span of patients with acute leukemia as a result of chemotherapeutic and adrenal hormone management, permitting leukemic cells in the meninges more time to proliferate; and (2) the currently used chemotherapeutic agents do not seem to gain access to the cerebrospinal fluid or the brain parenchyma in the same relative concentrations as in the serum and somatic organs so that the leukemic process may be better controlled generally than in the central nervous system [*49,50*].

Meningeal leukemia is associated with increased intracranial pressure [*49*]. The principal clinical manifestations are nausea and vomiting, headache, lethargy, convulsions, papilledema, cranial nerve palsies and other less specific neurological signs. A positive Kernig's sign is unusual. Skull suture lines are frequently spread in children. Examination of the cerebrospinal fluid reveals increased pressure, a pleocytosis of abnormal cells (25 to 11,000 per cu. mm.), or both. Cultures are negative. There is no constant relationship between the leukemic changes in the blood and bone marrow and those in the meninges; the patient may be in hematologic remission or in any stage of relapse. Evidence has been presented that the syndrome of meningeal leukemia is caused by arachnoidal infiltration, particularly along the brain stem, with resulting obstruction of flow of cerebrospinal fluid and consequent hydrocephalus [*51*]. In children, median survival after onset of meningeal leukemia has been reported to be three months (one-half to eleven months) [*49*]. Hyperphagia, pathologic weight gain and behavioral disturbances (hypothalamic syndrome) have occasionally been observed in association with leukemic meningitis and leukemic infiltration of the hypothalamus.

Intracranial hemorrhage has long been recognized as a not uncommon terminal event in patients in whom thrombocytopenia develops during the course of any form of leukemia. Recent studies have suggested that thrombocytopenic intracranial hemorrhage is predominantly subarachnoid although the hemorrhage frequently extends into the brain substance

[*51*]. Intracerebral hemorrhage (primarily into the cortex but with extension, when massive, into ventricles or the subarachnoid space) in patients with acute leukemia, however, is a distinctive intracerebral lesion with nodules of leukemic cells within the areas of hemorrhage [*51,52*]. Intracerebral leukostasis appears to result in localized proliferation, destruction of the vessel wall, formation of a leukemic nodule and hemorrhage about the nodule. Coalescence of many such lesions results in the large hemorrhages seen grossly. This complication occurs during blastic crises with marked elevation of the leukocyte count; the lesion was not found in any patient whose white blood cell level was below 100,000 per cu. mm. and was frequent when the count rose above 300,000 per cu. mm. By contrast, there was no correlation between the degree of leukocytosis and thrombocytopenic-related subarachnoid hemorrhage. In 117 consecutive patients with acute leukemia, intracranial hemorrhage was responsible for death in twenty-three; in these the hemorrhage was predominantly intracerebral in fourteen and subarachnoid in nine.

Anemia. Long experience and the work of many investigators have established that anemia occurs during the course of nearly all leukemic patients, and is caused by a variable combination of depressed erythropoiesis, accelerated hemolysis and hemorrhage [*3,12,53*]. Erythrokinetic and survival data have demonstrated that a hemolytic component is quite common, that the bone marrow in an attempt to compensate may often produce red blood cells at an accelerated rather than a depressed rate, but that the erythropoietic response is suboptimal and unable to keep pace with the increased hemolysis (relative marrow failure) [*54*]. The underlying mechanisms are still poorly understood. The result of the Coombs' test is positive in about one-third of anemic patients with chronic lymphocytic leukemia and rarely in anemic patients with other types of leukemia; in these instances, an "autoimmune" process may be of pathogenic importance. When patients respond satisfactorily to treatment, the red blood cell count frequently returns to or toward normal as a remission is established. There is one important exception, however, not sufficiently appreciated by most physicians: the anemia associated with chronic lymphocytic leukemia only rarely improves with antileukemic therapy *per se*. That is true whether the anemia occurs early in the disease, at the time of relapse from an induced remission, or appears when the leukemia is otherwise not producing any clinical manifestations. Exceptions do occur with Chlorambucil therapy, radiation to the spleen or with the administration of radioactive phosphorus, but they are clearly exceptions. For instance, Reinhard and his associates [*55*] found that among patients who responded well to treatment with radioactive phosphorus, only 9 per cent of anemic patients with chronic lymphocytic leukemia had a hemoglobin rise as great as 2 gm. per cent, and none had an increase of as much as 4 gm. per cent. Satisfactory to excellent therapeutic results, on the other hand, can often be obtained through the administration of adrenal steroids or by splenectomy [*3,56*]. More detailed discussion of therapy is not appropriate in this section of the symposium; the point to be emphasized here is that the anemia of chronic lymphocytic leukemia often behaves differently from the anemias associated with other forms of leukemia: it may appear during the course of a sustained remission or a quiescent phase, and it usually does not respond to ordinary antileukemic therapy. Whether the anemia is caused by a different pathogenic mechanism is not known, but the more frequent occurrence of a positive reaction to the Coombs' test is at least of interest.

Hemorrhagic Manifestations. Hemorrhage from mucous membranes, in the skin or into other organs and tissues is a frequent complication of all forms of leukemia, and vies with infection for the number one position as the most common cause of death. Hemorrhagic manifestations often comprise the presenting complaints in acute leukemia, and are found in 25 per cent of patients with chronic myelocytic and in 5 per cent of patients with chronic lymphocytic leukemia at the time of diagnosis [*18*]. All components responsible for adequate hemostasis, platelets, coagulation factors and capillaries, have been suggested as the site of the abnormality, but the pathogenesis of the hemorrhagic diathesis is not fully understood. No clear demonstration of primary capillary defects has ever been made. Thrombocytopenia in leukemia is ordinarily attributed to suppression of megakaryocytes. In some instances, however, particularly in chronic lymphocytic leukemia, a mechanism similar to that found in idiopathic thrombocytopenic purpura seems to be operative: megakaryocytes are plentiful in the bone marrow, platelet agglutinins may be found and a

good therapeutic response may be obtained with corticosteroid therapy or with splenectomy [*56,57*]. No precise correlation can be made between the degree of thrombocytopenia and the occurrence of bleeding. The platelet count may be low for weeks or months without hemorrhage; and then, even though no further change occurs, grave hemorrhagic manifestations may appear in explosive fashion. Explanation of this phenomenon is still not possible. Formation of plasma thromboplastin is diminished *in vitro* when the platelet count is below a level of about 60,000 per cu. mm. Recently, qualitative defects consisting of impaired ability to generate thromboplastin have been demonstrated in some patients with chronic lymphocytic, chronic myelocytic and acute leukemia [*58*]. Since excessive numbers of platelets may also inhibit the formation of thromboplastin, the high platelet levels occasionally found in chronic myelocytic leukemia may be responsible for the bleeding tendencies [*59*].

Quantitative deficiencies in the blood clotting factors are unusual in chronic leukemia. In acute forms of the disease, mild prolongation of the prothrombin time and a diminished level of proaccelerin are often found [*60*]; circulating anticoagulants and prolonged plasma thrombin times have occasionally been observed. Fibrinolysis and hypofibrinogenemia have been detected on rare occasions [*60–62*]. Although overt fibrinolysis is unusual, an increase in fibrinolytic activity in leukemic plasma is not unusual [*63*]. In some patients the presence of fibrinogen breakdown products, secondary to increased plasma proteolytic activity, has been observed in the circulation. These products interfere with the normal polymerization of fibrin and may contribute to the blood clotting anomalies described [*64*]. No constant correlation, however, has been made between hemorrhagic manifestations and any of the factors mentioned except for severe thrombocytopenia.

The distinctive type of intracerebral bleeding associated with blastic crises in acute leukemia [*51,52*] has already been described under "neurologic manifestations."

SURVIVAL

A significant clarification of our concepts about survival time in leukemia has resulted from Tivey's clear argument that duration of the disease should be expressed as "median survival time" rather than as average duration [*1*]. When per cent patient survival is plotted against time of survival, a skew curve results (Fig. 1) rather than a bell-shaped normal distribution curve, because of a few patients with a relatively long life span. Inclusion of these patients in an arithmetic mean could give an erroneous impression of duration. Tivey suggested that the difficulty could be overcome by plotting the cumulative per cent dead as the abscissa and the log of time as the ordinate on logarithm probability paper; a straight line results and a statistically analysable value, "the median survival time," may then be obtained by reading the time interval at 50 per cent survival. Many difficulties, however, remain, difficulties in obtaining suitable untreated control groups for comparison with treated patients, problems in classification and case selection, and variations in recording onset (first symptoms, time of diagnosis and time of beginning therapy).

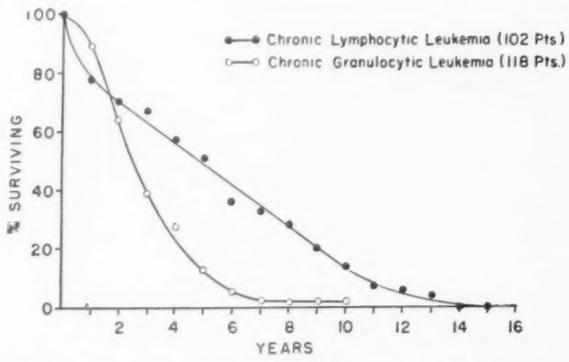


FIG. 1. Survival in chronic leukemia, per cent of patients surviving at yearly intervals after diagnosis. (From: Reinhard, E. H., Neely, C. L. and Samples, D. M. *Ann. Int. Med.*, 50: 942, 1959.)

Using his method, Tivey has carefully analyzed case reports of patients with acute leukemia treated only with palliative methods and transfusions. The median survival times from onset of symptoms to death in children were: acute granulocytic leukemia, 4.2 months; acute lymphocytic leukemia, 3.6 months; and acute monocytic leukemia, 2.6 months. The differences were not statistically significant among these groups, or between the survival of adults and children with acute leukemia. A similar analysis of thirty-two series of treated patients with chronic leukemia showed no difference in survival between chronic lymphocytic and chronic myelocytic leukemia (Fig. 2): 2.70 years for chronic granulocytic leukemia and 2.77 years

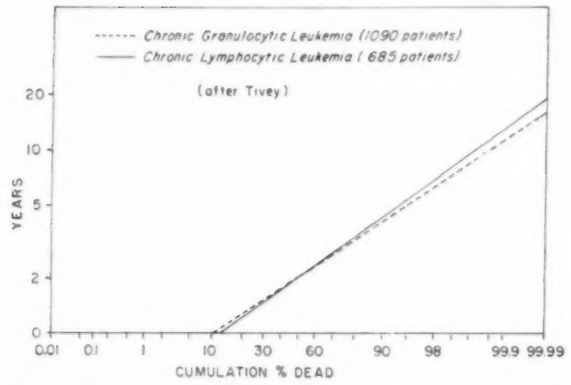


FIG. 2. Total duration from onset of symptoms to death of patients with chronic leukemia, after Tivey [*65*]. The cumulative per cent dead is plotted on logarithm probability paper against the log of time. The "median survival time" is the time at which there is 50 per cent survival.

for chronic lymphocytic leukemia [*65*]. Similar results have been obtained by other workers [*18*]. Certainly, chronic lymphocytic leukemia in the aged may follow a benign course; this fact has prompted the suggestion that it may in reality be a different entity from chronic lymphocytic leukemia in younger people [*3*]. It is of interest that Reinhard et al. [*55*] obtained a straight line for survival (plotted by Tivey's method) of patients with chronic myelocytic leukemia, but their data for chronic lymphocytic leukemia showed a significant departure from linearity. One possible interpretation would be that the latter group was heterogeneous, but they could find no correlation with age. They recorded a median survival time of 2.64 years for patients with chronic myelocytic leukemia treated with radioactive phosphorus; 12.5 per cent of patients with chronic granulocytic and 51 per cent of patients with chronic lymphocytic leukemia survived five years. Osgood and his associates reported the remarkably good median survival time of four years and four months for patients with both diseases grouped together, treated with regular titrated doses of radioactive phosphorus [*66*].

A few factors of prognostic significance will be listed. In acute leukemia, the presence of many blast forms usually indicates that the course will be rapid; survival tends to be longer in children with acute lymphocytic leukemia especially if the initial leukocyte level is less than 10,000 cells per cu. mm. [*67*]. The spontaneous remission rate in acute leukemia apparently varies from 0 to 10 per cent, and when it occurs has an average duration of 6.6 weeks [*1*]. Severe anemia, thrombocytopenia with hemorrhagic tendency, infection and skin involvement tend to be poor prognostic signs in chronic granulocytic leukemia. A similar statement except for the dermatologic manifestations may be made for chronic lymphocytic leukemia [*18*].

REFERENCES

1. TIVEY, H. The natural history of untreated acute leukemia. *Ann. New York Acad. Sc.*, 60: 322, 1954.
2. DOAN, C. A. and WISEMAN, B. K. Monocyte, monocytosis, and monocytic leukemia. *Ann. Int. Med.*, 8: 383, 1934.
3. DAMESHEK, W. and GUNZ, F. Leukemia. New York, 1958. Grune & Stratton.
4. WITTS, L. J. Environmental factors in human leukemia. In: The Leukemias, p. 151. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press, Inc.
5. World Health Organization Annual Epidemiological and Vital Statistics for 1956, pp. 25, 306, 350, 1959.
6. WAKISAKA, G. Clinical and statistical research of leukemia in Japan, especially in the Kinki district. *Acta haemat. Jap.*, (supp. 1), 21: 240, 1958.
7. SHIRAISHI, T. Clinical and statistical research of leukemia in Hokkaido over the 7 year period 1951–57. *Acta haemat. Jap.*, (supp. 1), 21: 259, 1958.
8. OTA, K. Clinical and statistical studies of leukemia in the Tokai district. *Acta haemat. Jap.*, (suppl. 1), 21: 279, 1958.
9. TING, N. and HWANG, T. Leukemias in Chinese armed forces. *Acta haemat. Jap.*, (supp. 1), 21: 371, 1958.
10. CHATTERJEA, J. B., GHOSH, S., RAY, R. N. and BANERJEE, D. K. Observations on leukemia in India. *Acta haemat. Jap.*, (supp. 1), 21: 314, 1958.
11. GRIEG, H. W. B. The incidence of leukemia in the Bantu of South Africa compared with the incidence in persons of European stock in South Africa. In: Proceedings of the Sixth International Congress. International Society of Hematology, p. 61. New York, 1958. Grune & Stratton.
12. WINTROBE, M. M. Clinical Hematology, 4th ed., p. 910. Philadelphia, 1956. Lea & Febiger.
13. MACMAHON, B. and KOLLER, E. K. Ethnic differences in the incidence of leukemia. *Blood*, 12: 1, 1957.
14. BLOCK, M., JACOBSON, L. O. and BETHARD, W. F. Preleukemic acute human leukemia. *J. A. M. A.*, 152: 1018, 1953.
15. MEACHAM, G. C. and WEISBERGER, A. S. Early atypical manifestations of leukemia. *Ann. Int. Med.*, 41: 780, 1954.
16. COLLINS, D. H. and ROSE, W. McI. The nature of anaemia in leukaemia. *J. Path. & Bact.*, 60: 63, 1948.
17. MOHLER, D. N. and LEAVELL, B. S. Aplastic anemia: an analysis of 50 cases. *Ann. Int. Med.*, 49: 326, 1958.
18. SCOTT, R. B. Leukaemia. *Lancet*, 1: 1053, 1957.

19. Garriga, S. and Crosby, W. H. The incidence of leukemia in families of patients with hypoplasis of the marrow. *Blood*, 14: 1008, 1959.
20. Bassen, F. A. and Kohn, J. L. Multiple spontaneous remissions in a child with acute leukemia. The occurrence of agranulocytosis and aplastic anemia in acute leukemia and their relationship to remissions. *Blood*, 7: 37, 1952.
21. Hayhoe, F. G. J. Diagnostic and nosological problems at the borderline of leukemia. The leukemias: etiology, pathophysiology, and treatment. In: Henry Ford Hospital International Symposium, p. 493. New York, 1957. Academic Press, Inc.
22. Moloney, W. C. and Lange, R. D. Leukemia in atomic bomb survivors. II. Observations on early phases of leukemia. *Blood*, 9: 663, 1954.
23. Weymouth, P. P., Delfel, N. E., Doell, R. J., Steinbock, H. L. and Kaplan, H. S. Nucleic acid content of the thymic cells of normal and irradiated C57BL mice. *J. Nat. Cancer Inst.*, 15: 981, 1955.
24. Silver, R. T., Utz, J. P., Frei, E., III and McCullough, N. B. Fever, infection and host resistance in acute leukemia. *Am. J. Med.*, 24: 25, 1958.
25. Keye, J. D., Jr. and Magee, W. E. Fungal diseases in a general hospital. *Am. J. Clin. Path.*, 26: 1235, 1956.
26. Forkner, C. E., Jr., Frei, E., III, Edgcomb, J. H. and Utz, J. P. Pseudomonas septicemia. *Am. J. Med.*, 25: 877, 1958.
27. Boggs, D. R., Frei, E., III and Thomas, L. B. Clostridial gas gangrene and septicemia in four patients with leukemia. *New England J. Med.*, 259: 1255, 1958.
28. Wall, R. L. The use of serum protein electrophoresis in clinical medicine. *Arch. Int. Med.*, 102: 618, 1958.
29. Wetherly-Mein, G. and Cotton, D. G. Portal fibrosis in acute leukaemia. *Brit. J. Haemat.*, 2: 345, 1956.
30. McIloanie, S. K. and MacCarthy, J. D. Hepatitis in association with prolonged 6-mercaptopurine therapy. *Blood*, 14: 80, 1959.
31. Tertelbaum, J. I., Wiener, J. and Desforges, J. F. A serologic and electrophoretic study of the malignant and proliferative disorders of the hemopoietic and reticuloendothelial system. *J. Lab. & Clin. Med.*, 53: 535, 1959.
32. Rundles, W. The leukemias: etiology, pathophysiology, and treatment. In: Henry Ford Hospital International Symposium, p. 503. New York, 1957. Academic Press, Inc.
33. Beard, M. F., Pitney, W. R. and Sanneman, E. H. Serum concentrations of vitamin B_{12} in patients suffering from leukemia. *Blood*, 9: 789, 1954.
34. Miller, A. and Sullivan, J. F. Electrophoretic studies of the vitamin B_{12} binding protein of normal and chronic myelogenous leukemia serum. *J. Clin. Invest.*, 38: 2135, 1959.
35. Jim, R. T. S. Serum gamma globulin levels in chronic lymphocytic leukemia. *Am. J. M. Sc.*, 234: 44, 1957.
36. Azar, H. A., Hill, W. T. and Osserman, E. F. Malignant lymphoma and lymphatic leukemia associated with myeloma type serum proteins. *Am. J. Med.*, 23: 239, 1957.
37. Mackay, I. R., Taft, L. I. and Woods, E. F. Clinical features and pathogenesis of macroglobulinemia. *Brit. M. J.* 1: 561, 1957.
38. Mackay, I. R. Macroglobulins and macroglobulinaemia. *Australian Ann. Med.*, 8: 158, 1959.
39. Schwartz, T. B. and Jager, B. V. Cryoglobulinemia and Raynaud's syndrome in a case of chronic lymphocytic leukemia. *Cancer*, 2: 319, 1949.
40. Craig, A. B., Waterhouse, C. and Young, L. E. Autoimmune hemolytic disease and cryoglobulinemia associated with chronic lymphatic leukemia. *Am. J. Med.*, 13: 793, 1952.
41. Shaw, A. B. Cryoglobulinaemia and lymphoid leukosis. *Proc. Roy. Soc. Med.*, 49: 115, 1956.
42. Larson, D. L. and Tomlinson, L. J. Qualitative antibody studies in man. III. Antibody response in leukemia and other malignant lymphomata. *J. Clin. Invest.*, 32: 317, 1953.
43. Ultmann, J. E., Fish, W., Osserman, E. and Gellhorn, A. The clinical implication of hypogammaglobulinemia in patients with chronic lymphocytic leukemia and lymphocytic lymphosarcoma. *Ann. Int. Med.*, 51: 510, 1959.
44. Hudson, R. P. and Wilson, S. J. Hypogammaglobulinemia and chronic lymphocytic leukemia. *J. Lab. & Clin. Med.*, 50: 829, 1957.
45. Onat A. and Cooper, T. Relation of bone marrow findings to serum protein changes in lymphosarcoma, chronic lymphocytic leukemia, and Hodgkin's disease. *Blood*, 15: 114, 1960.
46. Griffiths, L. L., Gilchrist, L., King, M. B. and Brews, V. A. L. Cryoglobulin in lymphoid leukosis. *Lancet*, 2: 1286, 1954.
47. Janeway, C. A., Gitlin, D., Craig, J. M. and Grice, D. S. "Collagen disease" in patients with congenital agammaglobulinemia. *Tr. A. Am. Physicians*, 69: 93, 1956.
48. Chaplin, H., Jr. Studies on the survival of incompatible cells in patients with hypogammaglobulinemia. *Blood*, 14: 24, 1959.
49. Shaw, R. K., Moore, E. W., Freireich, E. J. and Thomas, L. B. Meningeal leukemia: a syndrome resulting from increased intracranial pressure in patients with acute leukemia. *neurology*, in press.
50. Whiteside, J. A., Philips, F. S., Dargeon, H. W. and Burchenal, J. H. Intrathecal amethopterin in neurological manifestations in leukemia. *Arch. Int. Med.*, 101: 279, 1958.
51. Moore, E. W., Thomas, L. B., Shaw, R. K. and Freireich, E. J. The central nervous system in acute leukemia: a postmortem study of 117 consecutive cases, with particular reference to hemorrhages, leukemic infiltrations, and the syndrome of meningeal leukemia. *Arch. Int. Med.*, in press.
52. Freireich, E. J., Thomas, L. B., Frei, E., III, Fritz, R. D. and Forkner, C. E., Jr. A distinctive type of intracerebral hemorrhage associated with "blastic crisis" in patients with leukemia. *Cancer*, 13: 146, 1960.
53. Dacie, J. V. Haemolytic anaemia in leukaemia. In: The Haemolytic Anaemias, p. 331. New York, 1934. Grune & Stratton.
54. Moore, C. V. The concept of relative bone marrow failure. *Am. J. Med.*, 23: 1, 1957.
55. Reinhard, E. H., Neely, C. L. and Samples, D. M. Radioactive phosphorus in the treatment of

chronic leukemias: long term results over a period of 15 years. *Ann. Int. Med.*, 50: 942, 1959.

56. Reinhard, E. H. and Loeb, V., Jr. Dyssplenism secondary to chronic leukemia or malignant lymphoma. *J. A. M. A.*, 158: 629, 1955.
57. Harrington, W. J., Minnich, V. and Arimura, G. The autoimmune thrombocytopenias. In: Progress in Hematology, vol. 1. Edited by Tocantins, L. M. New York, 1956. Grune & Stratton.
58. Perry, S. and Baker, M. Coagulation defects in leukemia. *J. Lab. & Clin. Med.*, 50: 229, 1957.
59. Spaet, T. H., Bauer, S. and Melamed, S. Hemorrhagic thrombocythemia, a blood coagulation disorder. *Arch. Int. Med.*, 98: 377, 1956.
60. Lewis, J. H., Burchenal, J. H., Ellison, R. R., Ferguson, J. H., Palmer, J. H., Murphy, M. L. and Zucker, M. B. Studies of hemostatic mechanisms in leukemia and thrombocytopenia. *Am. J. Clin. Path.*, 28: 433, 1957.
61. Pisciotta, A. V. and Schultz, E. J. Fibrinolytic purpura in acute leukemia. *Am. J. Med.*, 19: 824, 1955.
62. Cooperberg, A. A. and Neiman, G. M. A. Fibrinogenopenia and fibrinolysis in acute myelocytic leukemia. *Ann. Int. Med.*, 42: 706, 1955.
63. Sawyer, W. D., Fletcher, A. P., Alkjaersig, N. and Sherry, S. Studies on the thrombolytic activity of human plasma. *J. Clin. Invest.*, 39: 426, 1959.
64. Fletcher, A. P. Personal communication.
65. Tivey, H. The prognosis for survival in chronic granulocytic and lymphocytic leukemia. *Am. J. Roentgenol.*, 72: 68, 1954.
66. Osgood, E. E., Seaman, A. J. and Koler, R. D. Results of fifteen year program of treatment of chronic leukemia with titrated regularly spaced total body irradiation with phosphorus 32 or x-ray. In: International Society of Hematology. Sixth International Congress, Boston, 1956. New York, 1958. Grune & Stratton.
67. Hart, A., Altman, S. J., Wintrobe, M. M. and Cartwright, G. E. The influence of chemotherapy on survival in acute leukemia. Comparison of cases treated during 1954 to 1957 with those treated during 1947 to 1954. *Blood*, 14: 824, 1959.

The Clinical Management of Leukemia*

ARTHUR HAUT, M.D.,† MAXWELL M. WINTROBE, M.D. *and* G. E. CARTWRIGHT, M.D.
Salt Lake City, Utah

LITTLE is known of the cause of leukemia and nothing of its prevention; it remains a fatal disease. Nevertheless, important practical advances in clinical management have been made which ease the situation of its unfortunate victims. It is now recognized that with palliative therapy symptoms may be relieved for months or years; this is especially true for patients with chronic leukemia but applies to those with acute leukemia as well. In some situations longevity can be extended and, perhaps of equal importance for most patients, hope is kept alive. For the last reason alone, if the physician approaches palliative therapy with some optimism and vigor, his treatment will be humane, regardless of whether or not the desired remission is obtained. Success in therapy must be judged by this criterion as well as by the more readily measured parameters of leukocytosis, anemia and longevity upon which we tend to dwell.

The two general spheres of therapy, pertaining to the somatic and the psychic sequelae of the disease, are closely interrelated. Little is gained by physical palliation if mental suffering is prolonged. At times, the physician must temper the system of treatment which relies upon chemical and physical agents in order to avoid imposing an undue burden on the patient. In some circumstances a shorter remission may have greater value to the patient than a somewhat longer remission, if the latter must be spent in a hospital, away from family, friends and familiar surroundings. The physician should embark upon palliative therapy with due regard for all of these considerations and their application to the individual at hand.

For all types of leukemia, the somatic problems can be divided into five general areas: (1) suppression of the leukemic cells in the blood, bone marrow and tissues; (2) prevention and control of hemorrhage; (3) control of infections; (4) relief of anemia; and (5) maintenance of appetite and weight. The principles governing treatment in the last four areas are common to all types of leukemia. On the other hand, the chemical and physical agents employed to suppress leukemic cells have a limited spectrum of usefulness and are "specific" in the sense that some of them are of value in only one type of leukemia. For this reason, the nomenclature will be reviewed before proceeding with a description of these agents and their application.

Nomenclature. We adhere to the policy of identifying the types of leukemia by descriptive terms which include the characteristic morphological cell type in the title. More than the duration of the disease, it is the morphological appearance of the blood and bone marrow and other clinical features which distinguish the chronic from the acute types of leukemia. This basis permits the applicable therapeutic agents to be selected with greatest efficiency.

Chronic lymphocytic leukemia refers to that usually indolent disease, occurring most commonly after the fourth decade of life, in which the predominant leukocyte in the blood, bone marrow and infiltrates of certain tissues is the small, mature-appearing lymphocyte. Ordinarily, we include only patients with leukocytosis. Cases termed by others "aleukemic lymphocytic leukemia," wherein lymphocytic infiltration may be present in tissues and bone marrow but lymphocytosis is not present in the blood, we include in the group "lymphosarcoma" and may treat in a different fashion than the lymphocytic leukemias, as defined.

Chronic myelocytic leukemia refers to that type of leukemia marked by granulocytic leukocytosis, predominantly of myelocytes and juvenile and segmented neutrophils. There are lesser numbers of promyelocytes and only occasional myeloblasts in the circulation. Splenomegaly, hepatomegaly and a hypercellular marrow, normal in appearance other than for an increased myeloid:erythroid ratio, are accompanying features. This disorder is to be differentiated from myelofibrosis, myelosclerosis, mye-

* From the Department of Internal Medicine, University of Utah, College of Medicine, Salt Lake City, Utah. Investigations by the authors to which reference is made in this article were supported by a research grant (CY-2349) from the National Cancer Institute and by a graduate training grant (2A-5098) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

† John and Mary Markle Scholar in Medical Science.

lophthisic disorders and other conditions of diverse etiology associated with extramedullary hematopoiesis.

Acute leukemia is recognized by the presence of an increased number, generally a predominance, of leukoblasts in the bone marrow and most often in the blood as well. Acute leukemia may advantageously be further classified according to the kind of leukoblast in the blood or marrow. Thus acute *lymphoblastic* leukemia refers to those instances in which the lymphoblast is found; acute *myeloblastic* leukemia refers to those cases in which the myeloblast is found. *Monocytic leukemia* refers to those types in which large numbers of monocytes and seemingly related forms accompany the leukoblasts in the blood. In the *Naegeli* type, described by Downey [*1*], variable numbers of myelocytes and myeloblasts are found while cells of the monocytic series, presumably derived from myeloblasts, make up 60 per cent or more of the leukocytes. In the rare *Schilling* type of monocytic leukemia larger cells, resembling monocytes and histiocytes in appearance, which possess a bizarre twisted nucleus, lacy chromatin and irregular cell borders are seen; these cells are presumed to be derived from the reticuloendothelial system. Monoblasts may also be found.

It is not uncommon to see instances of acute leukemia with variable numbers of cells resembling monocytes, generally comprising only a small proportion of all of the cells, together with larger numbers of myeloblasts. Eventually the appearance of the blood may be indistinguishable from the typical myeloblastic leukemia. Some hematologists might classify such cases as monocytic leukemia, others as mono-myeloblastic leukemia and still others as myeloblastic leukemia. Such differentiation is on uncertain ground and one wonders if it serves a purpose at this time. In clinical management these cases behave as does myeloblastic leukemia.

Despite the differentiating criteria which have been set down [*2*] there remain a small number of cases in which the leukoblast cannot be better classified, even by those especially interested and experienced; such cases may be termed undifferentiated acute leukemia. The therapeutic and prognostic implications of the various types of acute leukemia will be discussed later.

The general principles guiding therapy will be discussed first. Then, for each type of leukemia the chemical and physical agents of special value, their mode of application and their effect on morbidity and longevity will be described.

PRINCIPLES GUIDING THERAPY

Diagnostic and therapeutic measures should be adapted to caring for the individual as an ambulatory rather than as a hospitalized patient. Success of therapy is often enhanced by this approach although at times one may have to accept risks not ordinarily assumed in the treatment of curable or self-limited disease. Insofar as possible the emotional and financial burdens of hospitalization should be avoided early in the disease since the latter will inevitably be required later to control infection and hemorrhage, transfuse, and provide requisite medical supervision and nursing care when the patient is unable to remain ambulatory.

All the varied clinical manifestations of leukemia are attributed to the proliferation of the pathological leukocytes. Symptoms and signs usually subside or disappear when measures which suppress leukemic cells are effective. However, success is at best only temporary and often is incomplete. Accordingly, one must be prepared to deal with the clinical manifestations individually, especially before antileukemic therapy can exert its effect, but also later, when basic therapy no longer is of much value.

In leukemia, the two principal immediate causes of death are hemorrhage (often intracranial) and infection [*3,4*]. The risks of either occurrence are greatest when the leukemia is in relapse while they represent only a small hazard during remission. It follows that when the likelihood of inducing a remission is great, supportive therapy may tide the patient through the crisis; when the likelihood of inducing a remission is small, supportive therapy has little chance of more than momentary success. For the patient with the greatest likelihood of achieving a remission, skillful supportive care is most critical.

Hemorrhage. With regard to hemorrhage, it is generally true that the bleeding tendency can be ascribed to thrombocytopenia. In chronic myelocytic leukemia, in the initial stages of the disease and perhaps through most of the course, there may be thrombocytosis, or at least normal numbers of platelets in the blood. Early in the course of this disease, if thrombocytopenia occurs it may be the result of too much therapy, rather than too little. Later in the disease, thrombocytopenia may accompany other signs of relapse and this may be benefited by treatment. Ultimately, thrombocytopenia and other signs of relapse will not be aided by antileukemic therapy.

Thrombocytopenia may occur earlier in the course of chronic lymphocytic leukemia than in chronic myelocytic leukemia, although at an early stage it is generally not severe and not accompanied by serious bleeding. Antileukemic

agents sometimes exert a beneficial action with regard to thrombocytopenia but here, too, excessive therapy will aggravate the problem. It is in acute leukemia that therapy has the most salutary effect. Profound thrombocytopenia accompanied by widespread purpura and many other hemorrhagic phenomena may be relieved in as little as two or three weeks in an individual responding to chemotherapy. The benefit may be retained during a long remission. Before the effect of therapy can be realized, hemorrhage may be controlled by judicious use of local measures, such as direct pressure packs in the case of epistaxis. Topical application of thrombin may be tried. The liberal transfusion of fresh platelet-rich blood, employing the commercially available all-plastic donor and recipient sets to minimize *in vitro* damage of platelets, has been advocated [*5*]. Transfusion of platelet concentrates isolated from 1 unit or more of fresh whole blood has also been useful [*6*]. Both of these measures are limited in practical application by the difficulty with which large amounts of suitable fresh blood can be made available and the fact that even in a child 2 units or more of blood are often required to increase temporarily the number of platelets and alleviate hemorrhage. In the adult, the problem is proportionately greater. Even when all precautions are taken, the use of non-wettable surfaces, fresh blood and even polycythemic donors, the procedure has only limited clinical value. Nevertheless, we generally use it for whatever benefit it may convey. Satisfactory platelet substitutes have not been found [*7,8*]. Lyophilized platelet material and soybean phosphatides [*9*], which have been claimed to offer some promise, are still under investigation and are not considered suitable for general clinical use.

Infections. Infections pose a greater problem in a patient with leukemia in relapse than in other situations because the host defenses are impaired. Organisms which ordinarily have little pathogenic potentiality cause serious infections. Minor furuncles may spread, perirectal abscesses are prone to occur and are especially resistant to surgical and chemotherapy [*10*]; both pyemia and septicemia are common and serious complications.

Prophylactic administration of antibiotics, intended to avert infection, probably only results in a change of the host's bacterial flora so that antibiotic-resistant organisms, including both bacterial and fungi, predominate. These organisms in turn become the causative agents of ensuing infections. A study is now under way to determine if the net effect of prophylaxis is detrimental, as it has been presumed to be, or whether in conjunction with antileukemic therapy the number of infections may be reduced and survival increased. At present, however, even in the presence of marked neutropenia we advise against the administration of antibiotics except for the treatment of a specific infection.

As soon as infection is suspected, cultures, smears and other measures to identify the etiological agent should be taken. According to the apparent urgency of the situation, therapy may be begun even before the results of these studies are available. The appropriate modifications in treatment may be made when and if indicated. Preference should be given to the use of bactericidal rather than bacteriostatic agents whenever circumstances permit and greater than customary dosage should be used. The resistance of microorganisms to antibiotics is a relative matter. At times, the daily intravenous administration of a bactericidal agent such as penicillin, well tolerated even in amounts as large as 10 or 50 gm. (about 17 and 84 million units, respectively), may control an infection even though the microorganisms were judged "resistant" by the conventional antibiotic-disc sensitivity technic.

Anemia. The relief of anemia may be accomplished by antileukemic therapy alone, as long as the latter retains its effectiveness. In chronic myelocytic leukemia and in acute lymphoblastic leukemia, the initial antileukemic treatment often relieves anemia without the need for other measures [*11–13*]. This result may be achieved several times. In chronic lymphocytic leukemia [*12*] and in acute myeloblastic leukemia [*13*], the anemia is less likely to be helped. In all forms of leukemia, eventually the anemia persists and progresses despite antileukemic therapy. In this circumstance, as well as in the severely anemic patient at any stage of the disease or in the event of serious hemorrhage, transfusion must be considered. The decision to give a transfusion should not rest wholly upon the numerical value of the hemoglobin concentration or an equivalent measurement. Instead, the need for transfusion should be judged largely from observation of the patient's physiological adjustment to the degree of anemia present. As a guide, it should be recalled that it is rarely necessary to give a transfusion when the hemoglobin con-

centration is as high as 10 gm. per cent or more, except in the case of frank hemorrhage when transfusion may be given to prevent or combat shock. Transfusion may be considered also in instances of thrombocytopenia with continuous bleeding and oozing, when the platelet content ef fresh blood rather than the quantity of erythrocytes is important.

With increasing degrees of anemia, increasing numbers of patients will have symptoms of dyspnea, fatigue or perhaps angina pectoris sufficient to warrant transfusion if further restriction of activities is undesirable and a remission of the disease is not to be expected. In some individuals, especially but not exclusively in the younger group, anemia may produce few noticeable symptoms even when the hemoglobin concentration is less than 7 gm. per cent. We recall an eleven year old girl who participated in a state fair although her hemoglobin was less than 5 gm. per cent.

The decision to give a transfusion usually does not need to be made hastily. Often the adequacy of the physiological adjustment to moderate degrees of chronic anemia is better than either the physician or patient may have anticipated. Transfusion is not without hazards and these will be multiplied in the leukemic patient. Some reasons to avoid transfusion whenever possible are: the risks of homologous serum hepatitis and transfusion reactions, the possibility that after multiple transfusions it may become increasingly difficult to find suitable donor bloods and, finally, the cost and other implications of hospitalization for transfusion.

The administration of iron, liver extract or vitamin B_{12} is valueless except in the unusual circumstance in which anemia due to a specific deficiency state is present. Thus, iron deficiency may develop with repeated bleeding, for example, from menorrhagia.

The administration of folic acid may be detrimental [*14*]. In the event of a macrocytic or even megaloblastic anemia resulting from intensive therapy with a folic acid antagonist, the proper course is to interrupt administration of the antagonist temporarily, rather than to prescribe vitamin B_{12}, folic acid or citrovorum factor (folinic acid). The last compound may not only overcome the effect of the antagonist but may also accelerate the leukemia. The use of cobalt and various "hematinic" compounds and vitamins for "non-specific" benefit cannot be advocated. It may be stated here that there are no compounds capable of overcoming leukopenia, *per se*, other than agents which act upon leukemia in relapse when this, rather than the effect of chemicals or radiation, is the factor responsible for the leukopenia.

It has long been recognized that whole blood transfusion, particularly when fresh blood is employed, may induce a partial or even complete temporary remission in leukemia. It is uncommon, however, to see more than minor improvement after simple transfusion. Exchange transfusion in acute leukemia has been reported to bring about considerable improvement in half of the patients so treated and improvement in the bone marrow in up to 20 per cent of the cases [*15*]. This observation is of theoretical importance but finds little practical application since other means of treatment are more readily carried out and the remissions induced are more satisfactory and last longer than those associated with exchange transfusion.

General Well-Being. Appetite and weight are useful guides to the clinical result of therapy since they improve with suppression of leukemia. Corticosteroid therapy may result in bulimia and weight gain even without satisfactory antileukemic effect. On the other hand, side effects of x-ray therapy, particularly when the abdomen is radiated, as well as of some of the chemotherapeutic agents, may produce anorexia, nausea, vomiting and, if these are prolonged, weight loss. Certain drugs given in excessive amounts may produce stomatitis and ulceration of the oral mucous membranes, interfering with appetite and food intake in this manner. Advice on the selection of a diet for those with limited appetite and marginal nutrition and the avoidance of harmful side effects of therapy are sometimes the most important contributions the physician can make toward preserving the patient's appetite and weight.

AGENTS CAPABLE OF PRODUCING A REMISSION IN SPECIFIC TYPES OF LEUKEMIA

Chronic Myelocytic Leukemia. For nearly a century, means of inducing a remission in chronic myelocytic leukemia have been at hand. At various times since 1865, sodium arsenite, x-rays, benzol, and more recently radioactive phosphorus, nitrogen mustard, urethane, triethylene melamine (TEM), triethylenethiophosphoramide (thio-TEPA) and busulfan (Myleran®) have been advocated. While the newer agents may have the advantage of greater

convenience and fewer side effects, all have the same limitations: namely (1) pancytopenia, which is the main risk to the hematological system; (2) relapse of the disease following interruption of therapy; (3) lack of effectiveness in the acute myeloblastic phase; and (4) eventual loss of effectiveness of all therapeutic agents, with an inevitable fatal termination.

There is now a little evidence that the survival of groups of patients is increased by these agents [*16*]. The median survival of patients treated with radiation up to 1924 was twenty-nine months; for those treated with P^{33} up to 1950 it was variously reported at from thirty-two months to as much as forty-four months. It is still too early to fully evaluate busulfan in regard to survival although preliminary appraisal indicating a median survival of forty-two months [*17*] is encouraging. For individual patients it would seem that the treatment had interrupted rapid deterioration.

Symptomatic remission and objective improvement can be achieved with great regularity by treatment of chronic myelocytic leukemia [*4,11,12,18*]. Radiation and busulfan appear to be equally effective over periods of months or years [*19*]. Generally, patients may resume normal activities and often they exhibit little evidence of the disease for long periods of time. Eventually, it becomes exceedingly difficult to control the disease and then impossible. However, the time at which this may occur cannot be predicted when the patient is first seen. The period of well-being during therapy, if one considers all patients, extends over a remarkable range. Ten per cent of patients may survive only sixteen months; however, half may survive forty-two months, and 10 per cent may survive eighty-four months or more [*17*]. The choice between radiation or chemotherapy depends upon the physician's familiarity with the methods, the availability of facilities for radiotherapy or the handling of radioisotopes, and the practical consideration of the convenience to the patient with which radiation or chemotherapy may be administered and appropriate follow up arranged.

One risk encountered in the treatment of leukemia may be avoided by advance planning. The urinary excretion of uric acid has been found to be increased, as a rule, in all types of leukemia except chronic lymphocytic leukemia, in which the increase is inconstant and small [*20*]. When treatment is first begun, whether it be by radiation or chemotherapy, there may be a great increase in the urinary excretion of uric acid, exceeding the solubility of this material in the acid urine [*21*]. As a result there is danger of ureteral obstruction due to the precipitation of uric acid crystals [*22*]. The risk can be reduced by urging a high fluid intake. Whenever the number of leukocytes is being rapidly reduced from high levels, by therapy, and certainly when colicky or back pain together with a heavy sediment in the urine suggest impending obstruction, the pH of the urine should be increased by giving large quantities of alkali orally.

Radiation. Splenic radiation is frequently used. A number of apparently equally effective technics have been described. In one method [*23*] an initial dose of 25 r is administered, alternating between anterior and posterior 15 by 15 cm. splenic fields. The dose is gradually increased up to 100 or 150 r daily until the desired response has been achieved. Radiation is carefully graded to avoid leukopenia and thrombocytopenia. Over a period of three weeks, a total of 400 to 1,200 r may be administered. After several months, when relapse occurs, another course may be administered. In another technic [*24*] 50 to 100 r may be given daily over four to six days. After three to four weeks the full response appears. Additional treatment is then given if needed.

Spray radiation over the whole body has been especially advocated by Osgood and Seaman [*25*] who have claimed that a greater longevity can be achieved for patients treated in this way. Initially 10 to 25 r whole body radiation may be given once weekly. The dose is then repeated at regular interva's, the latter gradually increased to between four and twelve weeks, as necessary to keep the patient in remission.

Radioactive phosphorus (P^{32}) was introduced in the treatment of chronic myelocytic leukemia in 1936 by Lawrence [*18a*]. It may be given by the oral or intravenous route and is an alternative means of administering whole body radiation. It has the theoretical advantage of delivering proportionately more radiation to the marrow tissues [*18*] than to the rest of the body since the radioisotope is primarily incorporated into the nucleic acids of the most rapidly dividing cells, in this case the leukocytes. An additional advantage of P^{32} therapy compared to x-ray therapy is that the former rarely produces the side effects of nausea and vomiting after ordinary therapeutic doses. In one method of

administration [*18b*] 1 to 2 mc. is given orally or intravenously, once or twice weekly, for four to six weeks, to a total dose of 6 to 8 mc. The dose is regulated to avoid depression of erythrocytes and platelets. A remission is to be expected after the first course of therapy. Treatment is repeated when warranted by signs of relapse. At least six months should elapse between courses, and an arbitrary limit of 15 to 20 mc. per year has been suggested. Equally satisfactory results have been described from a different plan of treatment [*12*]. At fortnightly intervals, 1 to 2.5 mc. of P^{32} is administered intravenously, to a total of 6 to 12 mc. per course, in order to achieve a remission. Still another technic, "titrated regularly spaced total body radiation" with radioactive phosphorus [*25*], in which treatment is continued during remission, has been alleged to result in longer survival [*16*] than after other plans of treatment. One-half to 2.5 mc. of P^{32} is given intravenously, at first once weekly, then at increasing intervals, as determined from the individual's response. Maintenance therapy is given at four- to twelve-week intervals.

In prescribing radiation therapy, it is implicit that the dose be individualized to the patient's response. Overzealous therapy can produce aplasia of marrow. The variation of response in a group of twenty-seven patients with previously untreated chronic myelocytic leukemia required more than a tenfold dose range [*26*]. The amount of P^{32} required before the leukocyte count was halved varied from 0.55 mc. in the most responsive individual to 8.4 mc. in the least responsive one. One-fourth of the patients halved their leukocyte counts after receiving doses up to 2.2 mc.; another fourth responded in this way after receiving doses from 2.2 to 2.9 mc.; the one-fourth least responsive to P^{32} required from 4.5 to 8.4 mc. to accomplish the same result.

Chemotherapy. Of the number of available chemical agents, we consider busulfan to be superior because of the graded response that can be obtained and the few side effects; the results of treatment are equal to or superior to those that are obtained with other agents [*11,19,27*]. In our clinic, busulfan is generally prescribed for chronic myelocyte leukemia in preference to other chemotherapeutic agents and radiation.

About 60 μg./kg., generally amounting to 4 or 6 mg., is given daily in a single dose before breakfast. Treatment is continued until the number of leukocytes falls to below 10,000 per cu. mm., the duration of therapy being roughly proportional to the logarithm of the pretreatment leukocyte count. At these doses it can be anticipated that the leukocyte count will be halved about every three weeks. The total duration of therapy and total dose administered will depend upon the height of the initial leukocyte count and the depth of relapse when therapy was begun [*11*]. During treatment the patient is examined and the leukocyte count, differential, volume of packed red cells and platelet count are determined every two or three weeks. Following the first treatment the leukocyte count is generally reduced to normal, the differential count is normal, and anemia is relieved without having resorted to transfusion, the platelet count returns to normal and the spleen is either reduced to normal size or else to less than half its initial bulk. Remission may be maintained for periods up to two years, the longer remissions usually occurring in patients with a low leukocyte count when therapy was stopped [*17*]. During the period without therapy the patient may be seen at one- to three-month intervals, the exact time depending upon the course of events and the rate of rise of the leukocyte count. When the evidence of relapse is definite, treatment is begun again; by this time the number of leukocytes usually exceeds 50,000 per cu. mm. With repeated treatment any residual splenomegaly is further reduced. After subsequent courses of treatment the period of remission frequently becomes shorter [*17,28*]. When the leukocyte count rises from its value at the end of treatment, ideally 10,000 or less per cu. mm., to 50,000 per cu. mm. within three months, it is reasonable to consider maintenance therapy; i.e., 2 mg. of busulfan given two to three times weekly, or more often, as needed to prevent rapid relapse [*28*].

Eventually, regardless of the type of therapy, about half the patients enter a phase of the disease resembling acute myeloblastic leukemia [*4,17*]. Busulfan is not useful in this circumstance nor can much be expected from radiation. However, desacetylmethylcolchicine (demecolcin) [*29*] and 6-mercaptopurine (6-MP) may be of some value. A dose of 3 to 5 mg. daily of demecolcin may be tried. Often small changes in the dose may make the difference between no effect and over-response; an abrupt fall in leukocytes may occur. An equally abrupt rise may occur if administration of the drug is discontinued and, as a consequence, continuous

therapy is needed [*30*]. 6-Mercaptopurine is usually prescribed in a dose of 2.5 mg./kg. daily; one, two or more weeks may elapse before a definite result is obtained. Here, too, continuous therapy is needed to retain any benefit [*31*]. Both demecolcin and 6-MP have been advocated for use at any stage of chronic myelocytic leukemia, although we prefer to employ them only when busulfan fails to help.

Chlorambucil, usually employed in the treatment of chronic lymphocytic leukemia, has been reported to produce remissions in occasional patients with chronic *myelocytic* leukemia as good as [*32*] or nearly as good as [*33*] those produced by busulfan. While these observations are useful to dispel the notion of "specificity" of any one chemotherapeutic agent, it should be noted that with chlorambucil therapy of chronic myelocytic leukemia, relapse followed shortly after administration of the drug was discontinued, and that in a series of randomly assigned cases [*34*] the results with chlorambucil were inferior to those with busulfan.

Chronic Lymphocytic Leukemia. Chronic lymphocytic leukemia is often a slowly progressive disorder, the course of which may continue for six or more years in half the patients [*18*] and, in rare instances, for twenty years [*35,36*]. Patients in whom the disease is discovered "accidentally" may have the best prognosis [*18*]. It has been noted that patients may get along for months or years with only a few symptoms despite a considerable lymphocytosis, mild or moderate anemia, or some enlargement of lymph nodes or the spleen. Since therapy is but palliative, not too often capable of bringing about a full remission of all manifestations of the disease, but at the same time introducing the risk of adverse reactions, one must always carefully weigh the patient's symptoms together with the possible gains of therapy on the one hand and the risks and inconveniences of therapy on the other. In some cases for months or, rarely, years after the diagnosis is made it is not advisable to do more than observe and record the findings, reserving the therapy until signs or symptoms increase. Either radiation or chemotherapy may be used, or both, the choice depending upon the considerations stated earlier for chronic myelocytic leukemia.

Radiation. X-ray over the spleen may be given, although more cautious administration and smaller doses than previously described may be needed. Local radiation of enlarged node masses may be especially useful in chronic lymphocytic leukemia because lymphadenopathy may be the principal cause of symptoms. Osgood's technic of whole body spray radiation applies equally to myelocytic and lymphocytic leukemia.

P^{32} may be employed in a manner similar to that described for chronic myelocytic leukemia, although on the average the total dose needed may be smaller by as much as one-half [*12*]. Complete relief of anemia, or a substantial increase in the hemoglobin concentration, reduction in splenomegaly and relief of thrombocytopenia occur much less frequently than in cases of myelocytic leukemia in which this form of treatment is used [*12*].

Chemotherapy. The useful agents include chlorambucil, TEM, nitrogen mustard and the corticosteroid hormone, cortisone, together with the related steroid derivatives (prednisone, triamcinolone and dexamethasone). In addition, benefit has been described after the use of Thio-TEPA [*37,38*] and busulfan [*39*] but for chronic lymphocytic leukemia the latter drugs either offer no advantage [*31,40*] or else are inferior to other agents [*34*].

Chlorambucil is preferred. Given in a daily oral dose of 0.1 or 0.2 mg./kg. it affords a better regulated response with less chance of unexpected bone marrow depression from ordinary doses than does TEM [*27,33,41–44*]. Daily treatment is continued for weeks or months, the dose being adjusted according to the reduction of the lymphocytosis but avoiding thrombocytopenia, leukopenia or anemia as side effects of treatment. When no further decrease in lymph node enlargement or splenomegaly occurs and continued treatment would lead to cytopenias, administration of the drug is stopped. It may be resumed upon relapse. Other than the potential occurrence of bone marrow hypoplasia, toxic side effects after therapeutic doses have included dermatitis [*44*] and a case of jaundice and hepatomegaly [*45*] possibly attributable to the drug. Accidental ingestion of a single dose of 5 mg./kg. by a two and a half year old boy was followed by coma, convulsions and profound leukopenia but fortunately complete recovery occurred [*46*].

TEM may be given intravenously but its main advantage over nitrogen mustard is in the fact that it can be given orally [*47*]. An intravenous dose of 0.04 mg./kg. (2 to 3 mg. for the average adult) is said to have the therapeutic and marrow

depressant effect of 0.1 mg./kg. of nitrogen mustard [*48a*]. About 0.04 mg./kg. of TEM may be given intravenously or intramuscularly, daily for two to four days, without producing nausea or vomiting and a therapeutic effect may be realized [*48b*]. Use of the drug by the oral route has been criticized because of its irregular absorption. As a consequence, the response is unpredictable and the risk of marrow depression due to overdosage is increased. The response is more regular when the drug is taken in the fasting state together with 2 gm. sodium bicarbonate [*49*] to prevent the formation of the chemically reactive form, the ethylene immonium cation, in the acid gastric juice.

Together with bicarbonate, a dose of 2.5 mg. may be given; thereafter, the patient and his blood are examined once weekly, prior to succeeding doses, to determine if further treatment is indeed necessary and to avoid bone marrow depression from unnecessary continuation of therapy. Three or more weeks may elapse before the full marrow depressant action of a dose may be realized [*50*]. Depending on the response, in some cases the dose may need to be increased to 5 mg. weekly, while in other cases it may have to be decreased. Although nitrogen mustard is not often used in chronic lymphocytic leukemia because of the usual success with orally administered agents, it may be considered when there is widespread lymphadenopathy and the patient cannot be seen at frequent intervals. The dose-response relationship is fairly uniform and the record concerning the occurrence of side effects is better for nitrogen mustard than is the case with TEM. A total dose of 0.2 to 0.4 mg./kg. nitrogen mustard may be used. None of the aforementioned agents should be immediately preceded or followed by other alkylating agents or radiation therapy, as the effects of these on the bone marrow summate and pancytopenia might result.

The adrenal corticosteroid hormones are of special value for the control of frankly hemolytic anemias, especially those associated with a positive Coombs' test. Hemolytic anemia of this type occurs in about 10 [*18*] per cent of patients with chronic lymphocytic leukemia and may be greater in degree than the more common Coombs'-negative anemia of chronic lymphocytic leukemia; however, the response to treatment may be better in the former than in the latter. Forty milligrams of prednisone taken daily, divided into four equally spaced oral doses, is a suitable starting dose. In severe hemolytic states with preservation of the marrow's capacity for erythrogenesis, steroid therapy may be followed by improvement in just a few days. If a response does not occur, the dose of prednisone may be increased to 100 mg. or several hundred milligrams daily, depending upon the severity of the hemolytic process and the urgency of the situation. Once the rapid hemolysis has been arrested and the hemoglobin concentration has returned to normal, the dose of steroid may be gradually reduced in stepwise fashion, over a period of months. It is not uncommon for the hemolytic process to accelerate and anemia reappear if steroids are withdrawn abruptly. Sometimes, maintenance doses of 5 to 15 mg. daily of prednisone may be needed for months or years to prevent recurrence of a serious hemolytic process. In this circumstance, as we shall mention later, splenectomy may be considered. Steroids may also be of value when anemia other than the frankly hemolytic type is the main cause of the patient's symptoms and if other therapy fails to help, or if thrombocytopenia or leukopenia precludes the use of chlorambucil, TEM or radiation. In this situation the response of the anemia is usually not as good as that in the frankly hemolytic state. However, satisfactory improvement together with regression of splenomegaly and lymph node enlargement occur often enough to merit a trial. The well known side effects of steroids, especially with regard to infection, osteoporosis, likelihood of vertebral collapse and duodenal ulcer with possible hemorrhage or perforation are added risks to individuals who are already prone to infection, hemorrhage or vertebral collapse because of the basic disease or their age. For these reasons it is desirable to avoid prolonged use of these hormones when it is feasible to treat by other means. The concomitant administration of testosterone or other anabolic but less masculinizing hormones and, in postmenopausal women, the administration of estrogen as well, has been suggested to minimize osteoporosis. In addition, testosterone may have a stimulating effect on erythropoiesis [*18*].

Splenectomy, in a few situations, may be advisable but in the majority of cases it is to be avoided. Early in the disease, splenomegaly can be readily reduced by chemotherapy or radiation. Late in the disease, when the leukemia is refractory to such therapy, the patient is usually a poor surgical risk. In any event,

splenectomy is not curative at any time. Rarely, when the so-called symptomatic, frankly hemolytic anemia mentioned previously is not adequately controlled by steroids in patients who are satisfactorily regulated in regard to other aspects of chronic lymphocytic leukemia, splenectomy may be considered. It is almost always possible to arrest the hemolytic process if one is willing to increase the dose of steroids sufficiently. However, the side effects of steroids may limit their usefulness over long periods if large doses need be prescribed. At times surgery can be avoided by the conjoint administration of chlorambucil, TEM or nitrogen mustard with steroids. This may further reduce the hemolytic process, presumably by lysis of the leukemic tissue which is in some way responsible for the hemolysis.

The Acute Leukemias. The mainstay of palliative therapy in acute leukemia is chemotherapy. Radiation plays only a minor role and except for limited local application it is generally considered to be harmful rather than helpful. Nevertheless, experiments employing amounts of whole body radiation previously thought to be well above the lethal range are being conducted in conjunction with attempts to transfuse isologous bone marrow. These experiments will be reviewed and an appraisal of their clinical application will be made after chemotherapy has been considered.

Chemotherapy of acute leukemia differs in two ways from that used in chronic leukemia. First it assumes a primary role in management rather than being co-equal with radiation. Secondly, in acute leukemia there are three broad groups of chemotherapeutic agents which may be used and, while cross resistance occurs within each group, resistance does not develop between members of different groups until after an agent from each group has been used at least once. In chronic leukemia, when resistance develops this applies essentially to all chemotherapeutic agents and to radiation as well, although a somewhat better response may be obtained with one agent when the toxic actions of another limit its use. Because of these characteristics the scheme of palliative treatment for acute leukemia emphasizes the choice of the chemotherapeutic agent under a particular circumstance and employs a system of *continuous* treatment with a *succession* of agents. An agent is repeated only after all others have been used once. Remissions may be achieved or sustained by agents used for the second or third time although they seemingly had lost their effectiveness earlier.

The three main classes of agents are the steroids (cortisone, hydrocortisone, prednisone, prednisolone, methylprednisolone, triamcinolone and dexamethasone), the folic acid antagonists (aminopterin and amethopterin) and the purine antagonists (6-MP, 6-chloropurine and a host of other derivatives less well known and without advantage over the first two). Many schemes of management employing these agents have been described. Together with supportive therapy, including transfusions, antibiotics and careful medical supervision, they have made possible lengthening of the median survival of groups of children with acute leukemia from four and half to ten months [*51*] and, in the experience of some, to twelve [*52*] or more months. Ten per cent of these patients now survive more than eighteen months, compared to eleven months only a few years ago. Patients without leukocytosis at the start of therapy have the best prognosis. It is generally agreed that adults do not fare as well, with or without chemotherapy. This difference is, in part at least, attributable to the much more frequent occurrence of myeloblastic leukemia in the adult than in the child [*51*]. Accordingly, it is proposed that the current therapeutic agents are for the most part effective against lymphoblastic types of leukemia and are of only limited value in myeloblastic types. In fact, it has been suggested that the corticosteroid hormones, in the usual doses, may even be detrimental to the course of myeloblastic leukemia [*13*].

It has been suggested that combinations of the known active antileukemic agents may prove more effective than these agents given individually in succession [*53*]. Experimental therapy of transplanted leukemias in mice and observations concerning the emergence of resistant strains of bacteria exposed to antibiotics have been cited in support of this suggestion. Nevertheless, even if these systems were analogous to human leukemia, there is one practical deterrent to combination therapy: the toxic manifestations of two of the three classes of agents are similar. If toxic manifestations occur, the responsible agent can only be identified by stopping first one drug, then the other; in the course of this procedure there is an equal chance that the useful, rather than the offending agent, would be stopped first. Furthermore, if im-

provement is obtained, one cannot readily determine if benefit is attributable to one agent, the other, or to both together. However, after the chemotherapeutic agents, used one at a time, have become no longer effective, the combination of steroids with either a folic acid antagonist or purine antagonist may be tried since toxicity of these pairs would not be additive. It might be possible under these circumstances to obtain benefit when one drug alone produces only toxic signs.

We believe that an attempt should be made to classify the acute leukemias according to the morphological appearance of the leukoblasts in the untreated case. The important distinction is between the lymphoblastic type on the one hand and the myeloblastic and various monocytic types on the other. For the first group the prognosis is better and steroid therapy is usually of considerable value. For the second group the prognosis is worse and steroid therapy is not advised. We do not belabor the subclassification of the monocytic leukemias since they appear to fall into a single group for purposes of therapy.

In lymphoblastic leukemia the diagnosis can usually be made at an office visit and we generally initiate treatment with prednisone. For the average child and most adults, 40 mg. of prednisone per day, divided into four equally spaced oral doses will suffice. More precise dosage has not seemed necessary. Others have advised a dose of 0.30 to 0.46 mg./kg. in children [*54*]. It is better to give too much than not enough, since the dose is tapered and administration of the drug stopped by the sixth or eighth week of treatment, after the desired response has been obtained. It was suggested that with considerably larger doses of prednisone, 1,000 mg. daily or comparable doses of the related compounds, responses may be obtained in otherwise resistant cases, including a certain number of myeloblastic cases [*55–57*]. From most of these reports it is not possible to judge whether smaller doses would not have been as effective as the large ones. In the over-all groups the benefit does not appear to have been proportionately greater with large as compared with smaller doses, whereas the serious complications due to steroids were more frequent [*58*]. It would appear that doses of this magnitude, if they are to be used at all, would best be reserved for patients who are resistant to management with all the conventional drugs in the usual doses.

In lymphoblastic leukemia the response to steroids is generally quite uniform [*13*]. Remission can be expected to occur in from 70 to 90 per cent of cases. Usually, in the first week the leukocyte count falls, often to subnormal values, due to the disappearance of leukoblasts from the blood. In the third week the number of leukocytes rises again, but examination of the blood smear reveals that this can be attributed to a return of normal numbers of neutrophils. The hemoglobin usually begins to rise during the second week, shortly after the appearance of polychromatophilia of the erythrocytes. Ordinarily, transfusion can be avoided after this point for, as the remission progresses, the hemoglobin can be expected to rise to normal. Improvement in appetite and well-being, reduction in splenomegaly and lymphadenopathy, and decrease in purpuric phenomena usually occur by the second week of treatment. After four to six weeks maximum improvement is realized.

Generally after a total of six or eight weeks of steroid therapy, after maximum benefit has been obtained, administration of the second agent, 6-mercaptopurine (6-MP), is begun in the hope of sustaining the remission. The dose of the steroid is tapered and then stopped over a period of about one week. The dose of 6-MP is set at 2.5 mg./kg. adjusted to the nearest 25 mg. of the drug, and is taken once daily. Treatment with 6-mercaptopurine is continued daily until either a relapse occurs or signs or symptoms of overdosage are seen. This may mean continuous therapy for many months and, in some cases, years. One of our patients received the drug for thirty-one consecutive months, without ill effect, remaining in remission throughout. In some patients the initial stage of treatment with steroids may be omitted and treatment may be initiated with 6-MP. This would apply to patients with little anemia, no hemorrhagic signs, no fever and few of the systemic signs, for 6-MP is much slower than steroids in bringing about a remission. One to four weeks may elapse before the leukocyte count falls and blasts disappear from the circulation, while nine to twelve weeks may elapse before maximum benefit is realized. Often it may be necessary to give the patient a transfusion during this interval. It is the exceptional rather than the average patient who can be treated in this way. When applicable, this method has the advantage that only one agent is used to achieve a sustained remission, and steroids may be more effective when employed

in a later relapse than if they had been given initially as well.

The main toxic effect of overdosage of 6-MP is suppression of one or all the normal cellular elements of the marrow and blood. If this occurs administration of the drug may be stopped for a few days, then resumed at half the dose. Gradually, the dose may be raised somewhat to the maximum which avoids toxicity. However, a moderate leukopenia (3,000 to 4,000 leukocytes per cu. mm.) is frequently encountered in patients who sustain a long remission under treatment [*59*] and should not be considered sufficient ground to reduce the dose, for no harm comes from it. A lesser dose might permit the disease to escape from control earlier than would be the case if the standard dose were continued.

Another toxic effect of 6-MP is the development of jaundice. In our experience this is an infrequent occurrence but others have reported this complication in as many as one-third of the patients treated with 6-MP or the related compound, 6-chloropurine [*60*]. It is not always a simple matter to determine the etiology of jaundice in leukemic patients. The effect of the drug, homologous serum hepatitis, leukemic infiltration of the liver and the effect of serious infection must be considered. A diverse etiology is suggested by the observations that while jaundice generally clears when therapy with 6-MP is stopped, it may continue in some patients; in still other cases, jaundice may disappear although therapy with 6-MP is continued. Pathological evidence of liver damage attributed to administration of 6-MP is limited to "bile-stasis" [*61*].

Drug-induced cytopenia, attributable to overdosage, must be distinguished from that due to relapse of leukemia. While recurrence of leukoblasts in the blood may be a certain sign of relapse, the insidious development of anemia, thrombocytopenia or neutropenia may have the same implication. If these are accompanied by a return of sternal tenderness or enlargement of the spleen, relapse may be suspected; however, if these helpful signs are not found the distinction between overdosage, for which administration of the drug would be stopped, and relapse, for which an increased dose or a change to another drug would be the proper course, must be made by marrow examination. The finding of large numbers of leukoblasts would indicate relapse.

In the event of relapse it is better to modify therapy at the earliest sign than wait until there is marked deterioration of the clinical state. If the patient had been receiving less than 2.5 mg./kg. of 6-MP daily, the situation may be improved by increasing the dose to the standard level. However, if the patient had already been receiving the customary dose, we have not often found the disease brought into remission again by increasing the dose still more, for example to 3.5 or 5 mg./kg. Instead, that course may result in combining 6-MP toxicity with the clinical effects of a profound relapse. It is better to start therapy at once with the third agent, amethopterin or, if the clinical condition has deteriorated sharply, to return to steroids in doses larger than that used initially, to bring about another remission.

Amethopterin is the folic acid antagonist of choice, since dose regulation is more readily achieved than with aminopterin and a clinical superiority of dichloroamethopterin has not been clearly demonstrated. The daily dose of amethopterin varies from 1.25 mg. in a small child to 10 mg. in the large adult, with only a general relationship to body weight. The average child of two to eight years receives from 2.5 to 5 mg. daily, the exact dose being individualized according to the response. This drug used alone may bring about a complete remission in about 30 per cent of children with lymphoblastic leukemia, with lesser benefit in a larger proportion. Leukoblasts leave the circulation in a week or two if the desired effect is to be achieved, but it may take two months before maximum benefit occurs. For initial treatment and to sustain a remission, the dose may be increased if the response is suboptimal and reduced if symptoms or signs of toxicity occur. As with 6-MP, marrow suppression and cytopenias may be produced by the drug. In addition, macrocytic anemia and even the appearance of magaloblasts in the marrow may result from overdosage. Clinically, anorexia, nausea, vomiting and severe stomatitis with ulcerations of the buccal mucosa may be produced. Diarrhea may develop. Temporary alopecia may occur. These manifestations are best controlled by stopping administration of the drug for a few days when they are first detected rather than delaying, since once they occur they worsen rapidly. When these symptoms and signs have subsided, therapy is resumed at a smaller dose. Hepatic fibrosis and ascites have been described

after long term treatment with amethopterin [*62*].

The administration of amethopterin is continued daily until either toxicity or relapse occurs. The distinction is usually readily made from clinical signs and examination of the blood, but in some cases the marrow must be examined to differentiate these complications. The same considerations apply here as described for 6-MP. If, in the case of relapse, further deterioration occurs with upward adjustment of the dose, then still another course of steroids is given. Second, third or fourth remissions have been produced with these agents. An incomplete response at this stage may perhaps justify using so-called "massive" doses of steroids. If remission is achieved again, or at least if satisfactory improvement occurs, 6-MP may be given once more. The cycle of chemotherapy is continued from this point on, with more and more reliance upon transfusion to raise the hemoglobin; less satisfactory remissions are produced and there are more frequent infections. Thrombocytopenia is not fully relieved and, as a consequence, a greater number of bleeding problems occur. Eventually, infection or hemorrhage during a phase of uncontrolled relapse is responsible for the death of most patients.

The general plan of management for myeloblastic and monocytic leukemia is similar to that outlined for lymphoblastic leukemia, except in one important respect. In our experience steroid therapy has not been beneficial in most cases of myeloblastic leukemia, and may cause harm in some. Consequently, we do not prescribe steroids for the initial treatment. Instead, we begin treatment with 6-MP. Transfusions are generally required and full remissions do not occur often, but some relief is usually obtained. The same considerations apply here as in 6-MP therapy for lymphoblastic leukemia. Doses of 2.5 mg./kg. are continued daily until either relapse or toxicity occurs. In case of relapse, amethopterin is used next, the dose being the same as for lymphoblastic leukemia. If steroids are to be used at all, we advise reserving them until other agents have failed. The outlook in myeloblastic leukemia is not as good as in lymphoblastic leukemia. The median survival for treated patients is only seven months and few survive more than a year.

Other Agents for Acute Leukemia. Annually, thousands of compounds are subjected to screening procedures including *in vivo* tests against transplanted leukemia and other tumors in animals, in order to select those which may be of value in the chemotherapy of leukemia [*63*]. Only a few compounds pass the screen and are suggested for trial in man. Of these, most have been related to the chemotherapeutic agents already in general use. For the most part it has been noted that leukemia which is resistant to the effects of the parent antileukemic compound will also be resistant to the congener, and vice versa. As a practical matter, therefore, the addition of a new active congener only replaces another compound in the list of effective antileukemic agents and does not increase their number. Nevertheless, this process may permit identification of analogues with a greater separation between beneficial and toxic effects than is the case for the parent compound.

Some of these compounds may be reviewed here. At best, however, most of the compounds only equal the effectiveness of the agents now in general use. Some have not been studied sufficiently to be properly evaluated. Any of these might be given a trial when other measures fail.

Purine antagonists: 6-methylmercaptopurine (6-methylthiopurine); 6-mercaptopurine riboside; thioguanine (6-mercapto-2-aminopurine); thioguanosine (thioguanine riboside) [*64*]; 8-azaguanine (2-amino-8-azapurine) [*31*]. These compounds either offer no advantage over 6-MP or are inferior to it, and may be accompanied by side effects which outweigh any benefit produced.

The clinical response, side effects and toxicity of *6-chloropurine* are similar to those of 6-MP, except that the incidence of jaundice may be higher with the former [*60*]. Leukemia which is resistant to therapy with 6-MP is also resistant to 6-chloropurine. It would appear, therefore, from the reports available to date that there is no advantage in using any of these compounds in place of 6-MP.

Folic acid antagonist: Dichloroamethopterin (3′-5′-dichloro-amethopterin) has been reported to be more effective than amethopterin in prolonging survival in transplanted mouse leukemia [*65*]. Furthermore, the suggestion was made that in mice the new congener exerted some antileukemic action even though resistance to amethopterin had developed [*66*]. The toxic signs and symptoms are similar to those of the parent compound. This drug has been prescribed in an oral dose of 0.25 to 1 mg./kg. body weight, daily. It is currently being evaluated in

human leukemia. It cannot be recommended for general use until further information is available.

Glutamine antagonist: The serine analogue, 0-diazoacetyl-L-serine (azaserine) given alone to human subjects exhibits little antileukemic activity [*53*]. The suggestion had been made that given together with 6-MP it enhances the effectiveness of the latter [*67*]. A cooperative study in several treatment centers did not yield much support for this suggestion [*68*].

Pyrimidine analogues: 5-fluorouracil (5-FU) and 5-fluoro-2-deoxyuridine (5-FUDR). These compounds have been studied principally in solid tumors [*69*]. In a few children with acute leukemia resistant to therapy with 6-MP and amethopterin a complete remission occurred after the administration of FUDR. Both 5-FU and FUDR may produce stomatitis, bloody diarrhea, pancytopenia and hypoplasia of the bone marrow [*69*]. FUDR is thought to be less toxic than 5-FU at therapeutic doses. FUDR is given in intravenous injections of 30 mg./kg. per day for *five* days, with a maximum daily dose of 2,000 mg. [*70*]. If stomatitis, diarrhea or marrow depression do not occur, injections of 15 mg./kg. are given on the seventh and ninth days. A course of treatment such as this may be repeated after an interval of one to four weeks.

Compounds related to nitrogen mustard: Sarcolysine (L-phenylalanine mustard); BCM (mannitol mustard); aminouracil mustard; nitromin (methyl-bis-(β-chloroethyl)-amine-N-oxide hydrochloride); cytoxan (cyclophosphoramide). Some of these compunds can be given by the oral route. Other than this feature, they appear to offer as little as nitrogen mustard in the treatment of acute leukemia. Toxicity is similar to nitrogen mustard except that alopecia is common in patients treated with cyclophosphoramide.

Radiation Therapy in Acute Leukemia. Roentgen therapy of the skull may be used with benefit in chloroma and when central nervous system complications are produced by meningeal infiltration with leukemic tissue. Five to ten daily treatments of 50 r usually relieve symptoms and reduce the number of cells in the spinal fluid if these had been increased [*71*].

Total body radiation [*72,73*] has been tried experimentally in acute leukemia in man with the twofold purpose of (1) destroying all the leukemic tissue; and (2) altering the patient's tissue responses so that isologous bone marrow might be successfully grafted to replace the patient's marrow, destroyed by radiation. These experiments have been unsuccessful with the single exception of limited and temporary benefit to the leukemic member of a pair of identical twins, the normal twin acting as marrow donor.

Considerably more needs to be known before this technic can be generally recommended in therapy. The experimental studies have already directed attention to a number of problems, including host acceptance of isologous tissue, prevention and control of infection in a patient with profound leukopenia (less than 500 leukocytes per cu. mm.), and the relationship between the tolerable total dose of radiation and the rate of its administration. Our concepts regarding the effects of whole body radiation have been modified in two ways: first, the previously regarded range of "lethal" body radiation has been found to underestimate the tolerable dose, if the radiation is delivered over many hours rather than in a few moments; and, second, it has been shown that regeneration o the patient's marrow tissue can occur after weeks of hypoplasia following such a "supralethal" dose of radiation [*73*].

Concerning theoretical aspects of radiation therapy, two additional considerations should be kept in mind. Increasing evidence suggests that a viral etiology, already established in fowl leukosis, is probably operative in certain types of murine leukemia [*74,75*] and conceivably may be germane in the case of human leukemia [*76,77*]. The alleged virus particles of fowl leukosis have retained leukemogenic potential after 100,000 r radiation [*78*] while those from mouse [*75,77,79*] and human tissue [*77,78*] have not been inactivated by 50,000 r. If human leukemia is caused by a virus, its destruction by radiation seems unlikely since these exposures to radiation are considerably more than the supralethal doses so far used in human leukemia.

Secondly, the behavior of *transplanted* mouse leukemia is not identical with that of *spontaneous* mouse leukemias with regard to response to certain therapeutic agents [*80*]. The differences between transplanted mouse leukemia, generally used for animal studies, and spontaneous human leukemias may well be even greater than the differences between the two types of mouse leukemia; successes in treating transplanted mouse leukemia do not of necessity find their counterpart in the disease in man. Clinical failures with chemotherapeutic agents bear this out.

Special Problems in Management. In acute leukemia, an increasing number of instances of intracranial complications, attributed to leukemic infiltrations are being recognized [*81*]. These may develop and progress despite an apparent remission of disease elsewhere in the body. One explanation offered for this dichotomy serves as the rationale for the treatment of these complications. It has been observed in animals and in man that the potent antileukemic agents, amethopterin and 6-MP, do not readily diffuse into the spinal fluid and presumably their concentration in the meninges also is low [*82*]. It is hypothesized that antileukemic therapy which can keep the patient in remission prolongs his life and thus allows infiltration of leukemic cells into the meninges, unimpeded by chemotherapeutic agents in the milieu of the central nervous system. In treatment of this complication amethopterin may be given via the intrathecal route in a single dose of 0.1 to 0.5 mg./kg. body weight [*81–83*], the oral doses being interrupted for about twenty-four hours. Improvement in neurological manifestations may occur in four to eighteen days, whether or not the other signs of leukemia are in evidence or in abeyance. As already mentioned, x-ray therapy directed to the skull is another satisfactory means of treating this complication.

Pregnancy during the course of leukemia raises special problems. Transmission of the disease from mother to child does not appear to be a matter for concern, since there is only one recorded occurrence of leukemia during the first year of life in an infant born of a mother with leukemia [*84*]. However, fetal mortality is high and sometimes, but not always [*85*], there are added risks to the mother. Radiation and most of the chemotherapeutic agents, if administered during the first trimester of pregnancy, may result in abortion or developmental anomalies in the embryo. Even though there are isolated reports of the administration of busulfan for chronic myelocytic leukemia during pregnancy without fetal complications [*86,87*], this is not always the case [*88*] and these risks should be avoided if at all possible.

SUMMARY

Empirical systems of treatment, employing chemical agents and radiation in a manner specified for each type of leukemia, can relieve symptoms and offer the chance of an extended period of relative well-being to victims of leukemia. The use of whole blood transfusions and antibiotics, as the indications arise, is important if the best result is to be achieved.

Treatment of acute lymphoblastic leukemia frequently produces full remissions and, compared to untreated patients, at least a twofold increase in the duration of life after the onset of symptoms. In contrast, in the treatment of acute myeloblastic leukemia, neither full remission nor prolongation of life occurs frequently, although some benefit is sometimes obtained.

Therapy of chronic myelocytic leukemia may produce full remissions with great regularity; however, an accompanying prolongation of life has not been reported in most series. In chronic lymphocytic leukemia, remissions are often not as complete as in chronic myelocytic leukemia but symptoms are often less severe, the disease is more indolent and, in the experience of some, longevity is greater.

Although leukemia is inevitably fatal, for the untreated patient the period of suffering is longer, on the average, than for one who has been treated. In the end, the course of events, both in those who are treated as well as in the untreated, is likely to be trying for the patient, for his family and for the physician. Nevertheless, there are few families who regret that palliative therapy was undertaken.

REFERENCES

1. Downey, H. Handbook of Hematology. New York, 1938. Paul B. Hoeber.
2. Wintrobe, M. M. Clinical Hematology, 4th ed., p. 942. Philadelphia, 1956. Lea & Febiger.
3. Raab, S., Hoeprich, P. D., Wintrobe, M. M. and Cartwright, G. E. The clinical significance of fever in acute leukemia. *Blood*, to be published.
4. Scott, R. B. Leukaemia. *Lancet*, 1: 1053, 1099, 1162, 1957.
5. Freireich, E. J., Schmidt, P. J., Schneiderman, M. A. and Frei, E., III. A comparative study of the effect of transfusion of fresh and preserved whole blood on bleeding in patients with acute leukemia. *New England J. Med.*, 260: 6, 1959.
6. McGovern, J. J. Platelet transfusions in pediatrics. *New England J. Med.*, 256: 922, 1957.
7. Klein, E., Farber, S., Djerassi, I., Toch, R., Freedman, G. and Arnold, P. The preparation and clinical administration of lyophilized platelet material to children with acute leukemia and aplastic anemia. *J. Pediat.*, 49: 517, 1956.
8. Jackson, D. P., Sorensen, D. K., Cronkite, E. P., Bond, V. P. and Fliedner, T. M. Effectiveness of transfusions of fresh and lyophilized platelets in controlling bleeding due to thrombocytopenia. *J. Clin. Invest.*, 38: 1689, 1959.
9. Connor, W. E. and Carter, J. R. Influence of soybean phosphatide on blood coagulation and its use

in the thromboplastin generation test. *Proc. Soc. Exper. Biol. & Med.*, 97: 38, 1958.

10. Birnbaum, W. and Ahlquist, R. Rectal infections and ulcerations associated with blood dyscrasias. *Am. J. Surg.*, 90: 367, 1955.
11. Haut, A., Altman, S. J., Cartwright, G. E. and Wintrobe, M. M. The use of myleran in the treatment of chronic myelocytic leukemia. *Arch. Int. Med.*, 96: 451, 1955.
12. Reinhard, E. H., Neely, C. L. and Samples, D. M. Radioactive phosphorus in the treatment of chronic leukemias: long-term results over a period of 15 years. *Ann. Int. Med.*, 50: 942, 1959.
13. Fessas, P., Wintrobe, M. M., Thompson, R. B. and Cartwright, G. E. Treatment of acute leukemia with cortisone and corticotropin. *Arch. Int. Med.*, 94: 384, 1954.
14. Farber, S. et al. Action of pteroylglutamic conjugates on man. *Science*, 106: 619, 1947.
15. Bessis, M. and Dausset, J. Étude critique des rémissions au cours des leucémies aigues traitées par exsanguino-transfusions. *Rev. d'hémat.*, 5: 188, 1950.
16. Osgood, E. E., Seaman, A. J. and Koler, R. D. Results of 15-year program of treatment of chronic leukemias with titrated regularly spaced total body irradiation with phosphorus 32 or x-ray. In: Proceedings of the International Society of Hematology, (Sixth International Congress), p. 44. New York, 1958. Grune & Stratton.
17. Haut, A., Abbott, W. S., Wintrobe, M. M. and Cartwright, G. E. Busulfan in the long term therapy of chronic myelocytic leukemia. To be submitted for publication.
18. (*a*) Lawrence, J. H. The treatment of chronic leukemia. *M. Clin. North America*, 38: 525, 1954.
    (*b*) Lawrence, J. H., Dobson, R. L., Low-Beer, B. V. A. and Brown, B. R. Chronic myelogenous leukemia. A study of 129 cases in which treatment was with radioactive phosphorous. *J. A. M. A.*, 136: 672, 1948.
19. Galton, D. A. G. The use of myleran and similar agents in chronic leukemias. In: Advances in Cancer Research, vol. 4, p. 73. Edited by Greenstein, J. P. and Haddow, A. New York, 1956. Academic Press, Inc.
20. Cartwright, G. E., Sandberg, A. A. and Wintrobe, M. M. Uric acid excretion by patients with various types of leukemia. In: Proceedings of the Third National Cancer Conference, p. 357. Philadelphia, 1957. J. B. Lippincott.
21. Sandberg, A. A., Cartwright, G. E. and Wintrobe, M. M. Studies on leukemia. I. Uric acid excretion. *Blood*, 11: 154, 1956.
22. Kritzler, R. A. Anuria complicating the treatment of leukemia. *Am. J. Med.*, 25: 532, 1958.
23. Schlosser, J. V. Irradiation therapy of leukemia. In: Etiology and Treatment of Leukemia, p. 98. Proceedings of the First Louisiana Cancer Conference. Edited by Burdette, W. J. St. Louis, 1958. C. V. Mosby.
24. Karnofsky, D. A. The management of leukemia in adults. *New York State J. Med.*, 54: 3225, 1954.
25. Osgood, E. E. and Seaman, A. J. Treatment of chronic leukemias. *J. A. M. A.*, 150: 1372, 1952.
26. Easson, E. C. A quantitative study of the radiosensitivity of chronic leukaemia. *Brit. J. Radiol.*, 30: 35, 1957.
27. (*a*) Till, M. The use of chlorambucil (CB 1348) and busulfan (myleran) in the treatment of leukemia. In: Etiology and Treatment of Leukemia, p. 102. Proceedings of the First Louisiana Cancer Conference. Edited by Burdette, W. J. St. Louis, 1958. C. V. Mosby.
    (*b*) Schilling, R. F. and Meyer, O. O. Treatment of chronic granulocytic leukemia with 1,4-dimethanesulfonyloxybutane (myleran). *New England J. Med.*, 254: 986, 1956.
    (*c*) Turesson, D. Myleran treatment in chronic granulocytic leukaemia. *Brit. J. Haemat.*, 3: 220, 1957.
    (*d*) Unugur, A., Schulman, E. and Dameshek, W. Treatment of chronic granulocytic leukemia with myleran. *New England J. Med.*, 256: 727, 1957.
28. Galton, D. A. G. Treatment of the chronic leukaemias. *Brit. M. Bull.*, 15: 78, 1959.
29. Moeschlin, S. Round-table discussion on therapy. In: The Leukemias: Etiology, Pathophysiology, and Treatment (Henry Ford Hospital International Symposium), p. 645. Edited by Rebuck, J. W., Bethelland, F. H. and Monto, R. W. New York, 1957. Academic Press, Inc.
30. Leonard, B. J. and Wilkinson, J. F. Desacetylmethylcolchicine in treatment of myeloid leukaemia. *Brit. M. J.*, 1: 874, 1955.
31. Burchenal, J. H. and Ellison, R. R. Chemotherapy of human leukemia. In: Progress in Hematology, p. 265. New York, 1956. Grune & Stratton.
32. Krakoff, I. H., Karnofsky, D. A. and Burchenal, J. H. Remissions induced by chlorambucil in chronic granulocytic leukemia. *J. A. M. A.*, 166: 629, 1958.
33. Miller, D. G., Diamond, H. D. and Craver, L. F. A critical study of the clinical use of chlorambucil. *New England J. Med.*, 261: 525, 1959.
34. Rundles, R. W. et al. Comparison of chlorambucil and Myleran® in chronic lymphocytic and granulocytic leukemia. *Am. J. Med.*, 27: 424, 1959.
35. Moffitt, H. C., Jr. and Lawrence, J. H. Chronic leukemia of long duration: with a report of 31 cases with a duration of over five years. *Ann. Int. Med.*, 30: 778, 1949.
36. Tivey, H. and Moffat, C. A. The prognosis for survival in chronic granulocytic and lymphocytic leukemia. *Am. J. Roentgenol.*, 72: 68, 1954.
37. Shay, H., Zarafonetis, C., Smith, N., Woldow, I. and Sun, D. C. H. Treatment of leukemia with triethylene thiophosphoramide (thio-TEPA). *Arch. Int. Med.*, 92: 628, 1953.
38. Zarafonetis, C. J. D., Shay, H. and Sun, D. C. H. Triethylenethiophosphoramide in the treatment of chronic leukemia. *Cancer*, 8: 512, 1955.
39. Sykes, M. P., Philips, F. S. and Karnofsky, D. A. Comparative therapeutic activity of the nitrogen mustards and allied compounds. *M. Clin. North America*, 40: 837, 1956.
40. Ultmann, J. E., Hyman, G. A., Crandall, C., Naujoks, H. and Gellhorn, A. Triethylenethiophosphoramide (Thio-TEPA) in the treatment of neoplastic disease. *Cancer*, 10: 902, 1957.
41. Rottino, A. Therapeutic results in treatment of Hodgkin's disease with CB 1348 and R-48. *Blood*, 12: 755, 1957.
42. Bouroncle, B. A., Doan, C. A., Wiseman, B. K.

and FRAJOLA, W. J. Evaluation of CB 1348 in Hodgkin's disease and allied disorders. *Arch. Int. Med.*, 97: 703, 1956.

43. GALTON, D. A. G., ISRAELS, L. G., NABARRO, J. D. N. and TILL, M. Clinical trials of *p*-(DI-2-chloroethylamino)-phenylbutyric acid (CB 1348) in malignant lymphoma. *Brit. M. J.*, 2: 1172, 1955.
44. BETHELL, F. H., MEYERS, M. C., BISHOP, R. C. and SPENCER, H. H. Advances in the treatment of leukemia. *J. Michigan State M. Soc.*, 58: 614, 1959.
45. KOLER, R. D. and FORSGREN, A. L. Hepatotoxicity due to chlorambucil. *J. A. M. A.*, 167: 316, 1958.
46. WOLFSON, S. and OLNEY, M. B. Accidental ingestion of a toxic dose of chlorambucil. *J. A. M. A.*, 165: 239, 1957.
47. AXELROD, A. R., BERMAN, L. and MURPHY, R. V. Clinical and hematologic effects of triethylene melamine. *Am. J. Med.*, 15: 684, 1953.
48. (*a*) KARNOFSKY, D. A. et al. Triethylene melamine in the treatment of neoplastic disease. *Arch. Int. Med.*, 87: 477, 1951.
    (*b*) KARNOFSKY, D. A. Triethylene melamine in the treatment of lymphomas and leukemias. *M. Clin. North America*, 38: 541, 1954.
49. GELLHORN, A., KLIGERMAN, M. M. and JAFFE, I. Triethylene melamine in clinical cancer chemotherapy. *Am. J. Med.*, 13: 428, 1952.
50. SLIPYAN, A. Unusual toxicity to triethylene melamine in a case of chronic myelogenous leukemia. *Ann. Int. Med.*, 38: 331, 1953.
51. HAUT, A., ALTMAN, S. J., WINTRGBE, M. M. and CARTWRIGHT, G. E. The influence of chemotherapy on survival in acute leukemia. Comparison of cases treated during 1954 to 1957 with those treated during 1947 to 1954. *Blood*, 14: 828, 1959.
52. FARBER, S. Round-table discussion on therapy. In: The Leukemias: Etiology, Pathophysiology, and Treatment (Henry Ford Hospital International Symposium), p. 623. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press, Inc.
53. BURCHENAL, J. H. Treatment of leukemia. In: Etiology and Treatment of Leukemia, p. 109. Proceedings of the First Louisiana Cancer Conference. Edited by Burdette, W. J. St. Louis, 1958. C. V. Mosby.
54. HYMAN, C. B. and STURGEON, P. Prednisone therapy of acute lymphatic leukemia in children. *Cancer*, 9: 965, 1956.
55. GRANVILLE, N. B., RUBIO, F., JR., UNUGUR, A., SCHULMAN, E. and DAMESHEK, W. Treatment of acute leukemia in adults with massive doses of prednisone and prednisolone. *New England J. Med.*, 259: 207, 1958.
56. RANNEY, H. M. and GELLHORN, A. The effect of massive prednisone and prednisolone therapy on acute leukemia and malignant lymphomas. *Am. J. Med.*, 22: 405, 1957.
57. HILL, J. M. Round-table discussion on therapy. In: The Leukemias: Etiology, Pathophysiology, and Treatment (Henry Ford Hospital International Symposium), p. 629. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press, Inc.
58. GELLHORN, A. Round-table discussion on therapy. In: The Leukemias: Etiology, Pathophysiology, and Treatment (Henry Ford Hospital International Symposium), p. 635. Edited by Rebuck, J. W., Bethell, F. H. and Monto, R. W. New York, 1957. Academic Press, Inc.
59. HYMAN, C. B., BORDA-C., E. and DIGUMARTHI, G. What are normal blood values for leukemia in remission? *Blood*, 14: 369, 1959.
60. ELLISON, R. R., SILVER, R. T. and ENGLE, R. L., JR. Comparative study of 6-chloropurine and 6-mercaptopurine in acute leukemia in adults. *Ann. Int. Med.*, 51: 322, 1959.
61. MCILVANIE, S. J. and MACCARTHY, J. D. Hepatitis in association with prolonged 6-mercaptopurine therapy. *Blood*, 14: 80, 1959.
62. COLSKY, J., GREENSPAN, E. M. and WARREN, T. N. Hepatic fibrosis in children with acute leukemia after therapy with folic acid antagonists. *Arch. Path.*, 59: 198, 1955.
63. ENDICOTT, K. M. The chemotherapy program. *J. Nat. Cancer Inst.*, 19: 275, 1957.
64. HALL, B. E., WILLET, F. M., HALES, D. R., FEICHTMEIR, T. V., JERNER, P. W. and FRANCO, J. Experience with 6-methylmercaptopurine in the treatment of human acute leukemia. *Proc. Am. A. Cancer Res.*, 2: 210, 1957.
65. GOLDIN, A., VENDITTI, J. M., HUMPHREYS, S. R. and MANTEL, N. Comparison of the relative effectiveness of folic acid congeners against advanced leukemia in mice. *J. Nat. Cancer Inst.*, 19: 1133, 1957.
66. HUMPHREYS, S. R. and GOLDIN, A. Investigation of tumor variants recovered from mice with systemic leukemia (L-1210) after extensive therapy with 3′,5′-dichloroamethopterin and 3′-bromo-5′-chloroamethopterin. *J. Nat. Cancer Inst.*, 23: 633, 1959.
67. BURCHENAL, J. H., MURPHY, M. L. and TAN, C. T. C. Treatment of acute leukemia. *Pediatrics*, 18: 643, 1956.
68. FREI, E., III et al. A comparative study of two regimens of combination chemotherapy in acute leukemia. *Blood*, 13: 1126, 1958.
69. CURRERI, A. R., ANSFIELD, F. J., MCIVER, F. A., WAISMAN, H. A. and HEIDELBERGER, C. Clinical studies with 5-fluorouracil. *Cancer Res.*, 18: 478, 1958.
70. Cancer Chemotherapy Reports, No. 2, March 1959. Cancer Chemotherapy National Service Center, Bethesda, Maryland.
71. D'ANGIO, G. J., EVANS, A. E. and MITUS, A. Roentgen therapy of certain complications of acute leukemia in childhood. *Am. J. Roentgenol.*, 82: 541, 1959.
72. ATKINSON, J. B., MAHONEY, F. J., SCHWARTZ, I. R. and HESCH, J. A. Therapy of acute leukemia by whole-body irradiation and bone marrow transplantation from an identical normal twin. *Blood*, 14: 228, 1959.
73. THOMAS, E. D. et al. Supralethal whole body irradiation and isologous marrow transplantation in man. *J. Clin. Invest.*, 38: 1709, 1959.
74. GROSS, L. Viral etiology of "spontaneous" mouse leukemia: a review. *Cancer Res.*, 18: 371, 1958.
75. FRIEND, C. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exper. Med.*, 105: 307, 1957.

76. Dmochowski, L. et al. Studies on human leukemia. *Proc. Soc. Exper. Biol. & Med.*, 101: 686, 1959.
77. Schwartz, S. O. and Schoolman, H. M. The etiology of leukemia: the status of the virus as causative agent—a review. *Blood*, 14: 279, 1959.
78. Bergol'ts, V. M. An experimental study of the aetiology of the human leukaemias. II. The effect of treatment with formalin and glycerol, of high temperatures and radiant energy on the activity of human leukaemic factor. *Probl. gematol. i pereliv. krovi*, 4: 20, 1959. Abstracted in: *Problems Hemat. & Blood Transfusion*, 4: 19, 1959.
79. Schwartz, S. O., Schoolman, H. M. and Spurrier, W. Studies in leukemia. IX. The induction of leukemia in adult C3H mice by means of cell-free brain filtrates. *J. Lab. & Clin. Med.*, 53: 233, 1959.
80. Kirschbaum, A. Discussion—laboratory, clinical approaches to leukemia chemotherapy. In: Etiology and Treatment of Leukemia, p. 91. Proceedings of the First Louisiana Cancer Conference. Edited by Burdette, W. J. St. Louis, 1958. C. V. Mosby.
81. Cramblett, H. G. Recognition and treatment of intracranial manifestations of leukemia. *J. Dis. Child.*, 97: 805, 1959.
82. Whiteside, J. A. et al. Intrathecal amethopterin in neurological manifestations of leukemia. *Arch. Int. Med.*, 101: 279, 1958.
83. Murphy, M. L. Leukemia and lymphoma in children. *Pediat. Clin. North America*, 6: 611, 1959.
84. Cramblett, H. G., Friedman, J. L. and Najjar, S. Leukemia in an infant born of a mother with leukemia. *New England J. Med.*, 259: 727, 1958.
85. Sheehy, T. W. An evaluation of the effect of pregnancy on chronic granulocytic leukemia. *Am. J. Obst. & Gynec.*, 75: 788, 1958.
86. Izumi, H. M. Myleran in pregnancy, report of a case. *J. A. M. A.*, 161: 969, 1956.
87. Sherman, J. L., Jr and Locke, R. V. Use of busulfan in myelogenous leukemia during pregnancy. *New England J. Med.*, 259: 288, 1958.
88. Diamond, I., Anderson, M. M. and McCreadie, S. R. Transplacental transmission of busulfan (Myleran®) in a mother with leukemia. Production of fetal malformation and cytomegaly. *Pediatrics*, 25: 85, 1960.

Attempts at Transplantation of Human Bone Marrow in Patients with Acute Leukemia and Other Marrow Depletion Disorders*

FARID I. HAURANI, M.D. EVALYN REPPLINGER, M.B. *and* L. M. TOCANTINS M.D.
Philadelphia, Pennsylvania

THE demonstration that lethally radiated rodents can be protected by injections of bone marrow [*1*] and that certain forms of murine leukemia may be favorably influenced by total body radiation followed by injection of bone marrow [*2,3*] has stimulated much interest in the application of these findings to the treatment of leukemia and marrow depletion syndromes in man [*4*]. It may be said from the outset that, for reasons easily understood, the rigidly defined experimental conditions [*1*] have not been duplicated in man and may not be for some time. This may account for the fact that there is as yet no clearly demonstrated instance of a successful transplantation of bone marrow in human subjects.

Since experimental and theoretical considerations have already been adequately covered in previous publications [*5–7*], we shall discuss here only our own experience with the problems of procurement, processing and preservation of human bone marrow and its administration to patients with certain blood disorders.

MATERIAL AND METHODS

Procurement of Marrow. A successful "take" of marrow transplanted into experimental animals seems to depend on the extent of the attenuation of the host reaction (induced by radiation, antimetabolites or other suppressive agents) and the number of marrow cells injected [*8–9*]. It has been estimated that from thirty to fifty billion nucleated cells from human marrow are necessary for a successful transplant in man. Since the marrow employed in animal experiments was obtained from excised bones and the types of marrow cell responsible for recovery are not known, it has seemed advisable to use as many marrow cells as can be obtained, with a minimum admixture of blood. We have therefore worked almost exclusively with marrow from excised bones. After exploring various sources, the ribs and vertebral bodies proved to be the most suitable. The results of the procurement of such bones in a 1,200 bed teaching hospital in Philadelphia, during a forty-one-month period are shown in Table 1. Considering the quantity and availability for human use, ribs resected from patients without a malignant or infectious disorder (e.g., diaphragmatic hernia) have been the best source of marrow, next to ribs removed from voluntary healthy donors. Cadavers of persons recently deceased from non-infectious, non-neoplastic disorders may eventually be the best sources, when the now pressing problems of prompt availability of the cadaver and low temperature storage of the marrow have been solved. Because of these still existing

TABLE 1
MARROW OBTAINED FROM VARIOUS SOURCES

Source	No. of Subjects	No. of Bones
Ribs		
Voluntary healthy donors	22	42
Surgical specimens from patients with		
Malignant disease	196	218
Infectious disease	125	136
Non-malignant and non-infectious disease	171	199
Total	514	595
Vertebral bodies (obtained at autopsy)		
Patients with neoplasms	6	63
Patients with infections	2	27
Non-neoplastic, non-infectious	11	89
Total	19	179

* From the Charlotte Drake Cardeza Foundation, Jefferson Medical College, Philadelphia, Pennsylvania. This study was aided by a grant, AT(30-1)-1982, from the Division of Biology of the Atomic Energy Commission.

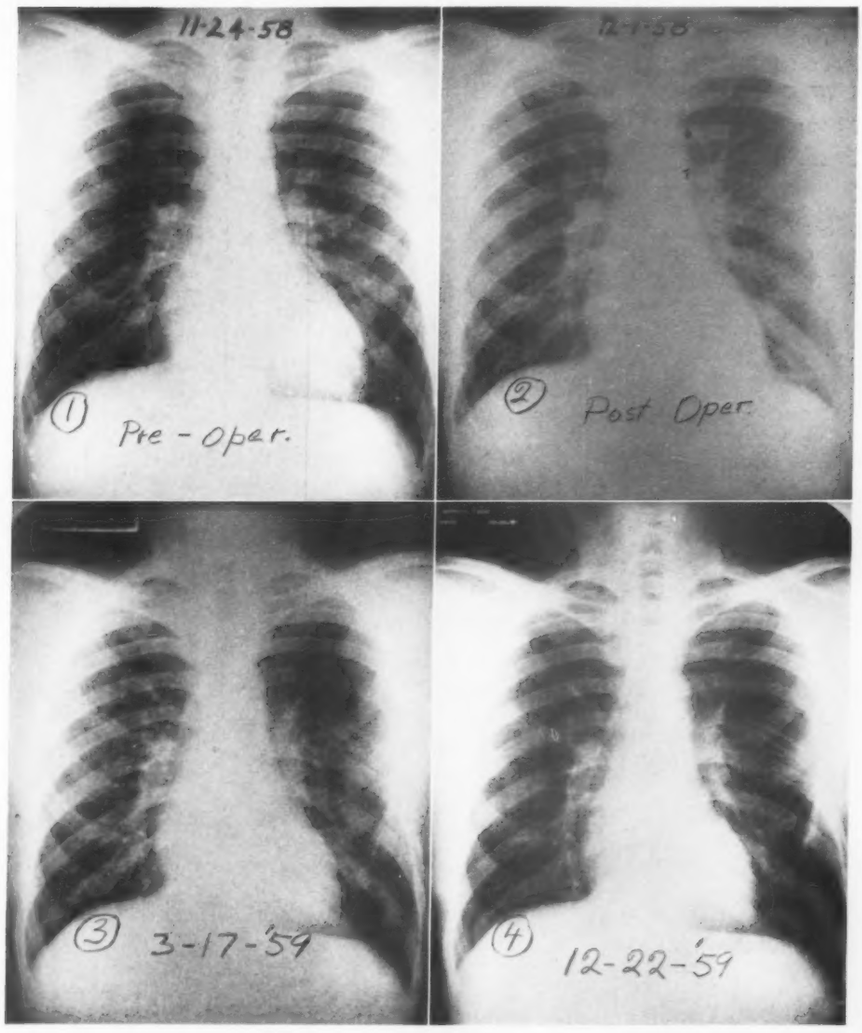


FIG. 1. Roentgenograms of the chest of a donor, of two ribs (left sixth and seventh) before and at various periods after resection. After one year both ribs were at an advanced stage of regeneration.

limitations marrow from the cadaver has not as yet been used by us for transplantation.

Processing of Material. Ribs: The fifth, sixth or seventh ribs are most commonly removed. No more than two ribs, from one side only, are resected from a single donor. Aseptic technic is followed throughout the procedure, from the time the rib is resected to the preparation and administration of the suspension of marrow.

The rib is thoroughly cleaned with a Bard-Parker knife and gauze sponges to remove all muscle, connective tissue, cartilage and adherent blood. An estimate of the amount of phosphate buffer 0.1 per cent sequestrene solution (P.B.S.)* necessary for the first leaching of the bone marrow is made on the basis of the length of the rib, its thickness and the amount and type of marrow present. Some ribs are quite dry and contain sparse marrow, others have large quantities of fat. Between 2 and 2.5 ml. of solution per inch of rib is used for the first leaching. This estimated volume is placed in a 600 ml. siliconized glass beaker with a lid. The rib is then carefully broken into pieces about 2 cm. long with a bone forceps, exposing the medullary cavity so that the cells may float out after the bone chips are dropped into the beaker. A direct smear of the undiluted bone marrow is made on a slide. The lid is replaced on the beaker and with gentle rotation the bone marrow cells are leached out of the bone chips. The supernatant is gently aspirated with a Pasteur pipette, placed in a 250 ml. graduated cylinder and stoppered. Cell counts and smears are made from aliquots of this suspension. A second

* Composition of P.B.S.: NaCl, 8 gm.; KCl, 0.2; NaH_2PO_4, 0.15 (anhydrous); K_2HPO_4, 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; Sequestrene (NA_2), 1.0; q.s. ad to 1,000 ml. with distilled H_2O.

TABLE II

TOTAL NUCLEATED CELLS IN SUSPENSIONS OF MARROW FROM EXCISED BONES

Source	No. of Cells
A. Ribs	
Maximum number of cells per rib	$5,443 \times 10^6$
Minimum number of cells per rib	64×10^6
Mean cell count per rib	$2,780 \times 10^6$
B. Vertebral bodies	
Maximum number of cells per vertebral body	$9,866 \times 10^6$
Minimum number of cells per vertebral body	$3,438 \times 10^6$
Mean cell count per vertebral body	$6,411 \times 10^6$

leaching is accomplished by adding fresh sequestrenated P.B.S. to the beaker of bone chips. Approximately 93 per cent of the cells are removed by three leachings, which are then pooled. The total nucleated cell count is estimated on the basis of the volume of the suspension and the cell count of a sample. Culture media are inoculated with aliquots of the marrow suspension. The processing must be swiftly carried out with as little agitation and handling of the suspension as possible to avoid injury of the cells. The suspension is passed once through two thicknesses of cotton gauze to remove any coarse bone particles. The total number of nucleated cells obtained from excised ribs used for transplantation in our patients are listed in Table II. The maximum yield represented rib marrow from a donor who had been primed by three venesections (once weekly) beginning one month before the rib donation. Six of the twenty-two normal healthy donors were available for follow up. Except for temporary discomfort, the donors had no immediate or remote ill effects from the operation.* Upon roentgenographic study, all had evidence of rib regeneration and are in good health. The extent of regeneration of the ribs in one donor thirteen months after resection is illustrated in Figure 1.

Processing of Marrow from Vertebral Bodies. This seems to be the most promising source of marrow for future attempts at transplantation. It is obtained at autopsies performed under special conditions, but has not been used in our patients. Thirteen to fourteen vertebral bodies may be taken from the thoracic and lumbar areas without causing a flail back. After evisceration of the cadaver, the nucleus pulposus is cut through with a hand saw at the desired upper and lower levels. The pedicles of the vertebrae are then sawed through (Fig. 2), first along one side of the column and then along the other. This will free the

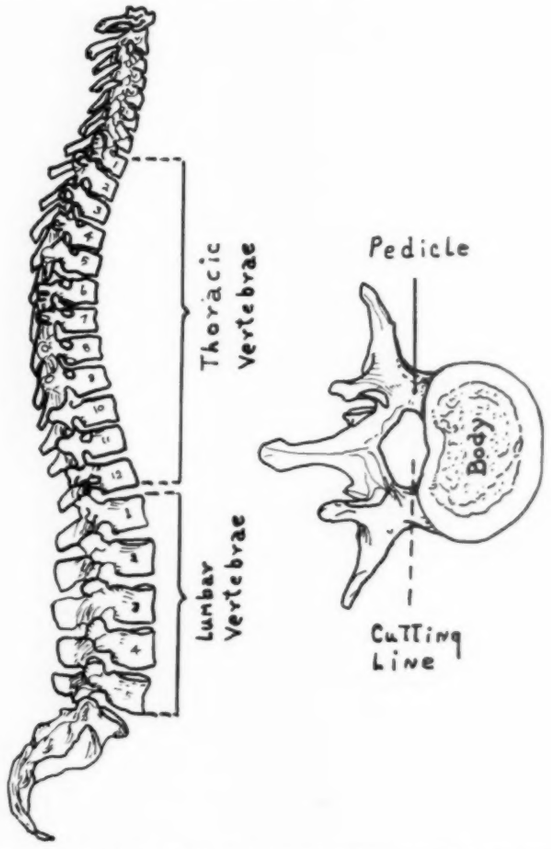


FIG. 2. Portions of the vertebral column useful for removal of marrow. By sawing through the pedicles at the points indicated, the vertebral bodies may be removed in one block.

vertebral bodies in one intact column, exposing the spinal cord, leaving the hematopoietic centers encapsulated in a bony case and allowing removal of the vertebral bone without previous contamination of the marrow itself. The removed column is liberally washed with tincture of Zephiran® and rinsed with sterile saline solution. Aseptic technics are observed throughout the entire procedure. Using the cutting blade of the Stryker hand saw, the column is split sagittally. The bone marrow is taken out in small scooping cuts and placed in a 600 ml. siliconized glass beaker containing 0.1 per cent sequestrene in P.B.S. The rest of the procedure is identical with that described for rib marrow.

Aspiration of Marrow/Blood Mixtures. This is a popular method of obtaining marrow from multiple punctures of bones such as sternum, ribs, anterior and posterior iliac crests. Aside from the fact that this method represents a radical departure from the methods utilized in animal experiments, the type and number of nucleated cells so obtained is disappointing. (Table III.) Moreover, the recent demonstration [*10*] of the deleterious effect of mixing blood with homol-

* Dr. John Y. Templeton, Department of Surgery, Jefferson Medical College, performed the rib resections in all the donors and made available to us ribs removed from patients undergoing thoracotomies.

TABLE III

COMPARISON OF THE MEAN PERCENTAGE COMPOSITION* OF MARROW REMOVED FROM EXCISED RIBS WITH THAT ASPIRATED FROM THE MARROW CAVITY

Source of Marrow	Erythropoietic Cells (%)				Myelopoietic Cells (%)							
	Pro-normo-blasts	Normo-blasts	Myelo-blasts	Pro-myelo-cytes	Myelo-cytes	Meta-myelo-cytes	Segmented Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils	Mega-karyo-cytes
Excised ribs	2.21	29.2	1.86	2.82	15.7	35	11.2	1.5	0.15	0.3	0.1	0.3
Aspiration	1.2	13.3	1.6	2.5	12.5	22.7	30	13	0.8	1.1	0.5	0.04

* Figures are means of counts on five specimens in each category. Only 1 ml. of marrow from the anterior iliac crest obtained at each aspiration.

ogous bone marrow for injection into radiated animals would seem to make aspirated bone marrow (with its large content of peripheral blood) undesirable in homologous marrow transplants in man. This point should not, however, militate against its use in autologous transplants.

Cell Viability. After the marrow cell suspension is prepared, a drop is inserted between a silicone coated cover slip and slide, and examined in a warm (38°C) chamber on the stage of a phase contrast microscope. The morphology, Brownian movement of cytoplasmic granules, ameboid and phagocytic activity are noted. Megakaryocytes and immature marrow cells may be readily identified. Fifteen $\times 10^6$ cells of the marrow suspension are incubated in 5 ml. of Billen's culture medium* "E" [*11*] at 37°C. for eighteen hours. A change in pH to the acid side (phenol red indicator) is assurance of the presence of cell respiration. Although none of these criteria supply any indication as to the "takeability" of the marrow, they do give some idea as to how far the manipulations involved affected the marrow cells.

Administration. Aseptic precautions are utilized throughout. With a Pasteur pipette most of the fat is removed from the surface of the marrow suspension. While the preparation is gently rotated to maintain the cells in suspension, the marrow is aspirated into a 50 ml. siliconized syringe. The suspension is then slowly (10 ml. per minute) injected into the tubing of an intravenous physiological saline infusion set, previously inserted into an arm vein. The syringe (with an eccentric tip) is held in such a manner that any remaining fat floats to the top of the suspension. Since the needle is inserted into the tubing and not into a vein, the syringe may be rotated to keep the suspension homogenous. The patient is closely observed for any unusual changes. There have been no immediate or delayed reactions after any of the injections given.

Technic of Total Body Radiation. Nine patients received radiation before the injection of marrow. The technics of treatment varied in each case, but the total dose was computed from depth-dose data or measurements in a phantom or both. Seven patients (Cases 3, 7, 10, 11, 12, 14 and 15) received total body radiation by the following technic:* The machine was a Westinghouse 250 kv, with output of 43 r per minute at 50 cm. or 4.7 per minute at 150 cm. It has an inherent tube filtration 3 mm. of Al and a filter of 2 mm. of Cu plus 1 mm. of Al added, with a resulting half value layer of 2.4 mm. Cu. A lead tunnel with a one-sixteenth inch lead was placed over the segments not being treated. Determination of the field size resulted from the maximum divergence of the beam which measured 48 cm. at the surface of the patient. The basic plan of treatment consisted of limited field exposures of 50 cm. t.s.d. along the spine and over the pelvis with a measured dose of 100 r in air to each field. Immediately after, within an over-all period of two to four hours depending on the number of segments treated, the 150 cm. distance from the mid-plane of the body was used, and 200 r in air was delivered to the total body with the patient in the prone position. Four patients (Cases 7 [second course], 14 [second course], 8 and 9) were given total body radiation with a Cobalt beam teletherapy machine† at a distance of 3.5 to 4 meters from the source to the skin, one-half dose through the anterior surface and one-half dose through the posterior surface, while the patient lay in a curled up position. The specified dose is a minimal measured dose at the mid-plane of body.

* AB, Rh + serum from human subjects substituted for horse serum.

RESULTS

The results have been arranged in three groups for analysis: Group 1, total body radiation of patients with acute leukemia followed by

* Carried out under the direction of Drs. Gerald J. Jerry and A. A. Mazzeo, St. Luke's Hospital, Newburgh, New York.

† Carried out under the supervision of Drs. Simon Kramer and Joseph Concannon, Department of Radiology, Jefferson Medical College Hospital, Philadelphia, Pennsylvania.

TABLE IV

PATIENTS WITH ACUTE LEUKEMIA WHO HAD TOTAL BODY RADIATION AND INTRAVENOUS INJECTIONS OF BONE MARROW FROM EXCISED RIBS

Case No.	Patient	Sex and Age (yr.)	Radiation Dose (r)	Nucleated Marrow Cells ($\times 10^6$)	No. of Ribs	Fluid Volume (ml.)	Survival Time after Marrow Injection
3	M. D.	F., 16	50	5,443	1	60	7 mo.
			300	18,573	4	353	14 days
7	S. W.	M., 4½	300	7,818	2	162	8 mo.
			600	9,494	4	300	23 days
8	J. M.	M., 59	350	4,317	2	90	18 days
9	H. L.	M., 24	350	8,550	2	78	2 mo.
10	L. B.	M., 12	350	6,451	2	175	4 hr.
11	J. B.	M., 4½	300	5,112	2	173	26 days
13	L. B.	M., 31	300	5,668	2	172	8 mo.
14	T. K.	M., 7	325	7,377	2	106	10 mo.
			502	29,646	10	670	13 days
15	S. S.	M., 4	300	5,831	2	166	12 days

injection of marrow. Group 2, injection of marrow in patients with aplastic and hypoplastic states. Group 3, injections of marrow following the use of marrow depleting drugs (HN_2, antifolic, antipurine compounds) as therapy for malignant lymphomas or leukemias.

Group 1. Among the different agents that have been used with the hope of paralyzing the immune mechanism of the host to prepare the way for a "take" of an infused homologous bone marrow, corticosteroids and total body radiation have seemed to be the most promising. Both of these agents were used in our attempts with the hope of paralyzing temporarily the immune host reaction to the transplant, while attenuating the leukemic process and possibly creating a chimera (donor's marrow and recipient's marrow functioning side by side) in which the donors marrow might prove to be resistant to the leukemic process.

Nine patients (four to fifty-nine years of age) with acute leukemia received twelve trials of total body radiation prior to infusion of bone marrow. (Table IV.) Three patients (Cases 3, 7 and 14) had two courses separated by a period of seven to ten months. Three patients (Cases 3, 13 and 14) were in hematological remission at the time of the procedure; their continued improvement for seven, eight and ten months, respectively, was probably unrelated to the radiation and infusion of bone marrow. One patient (Case 7), who was in relapse at the time he was first treated with radiation, had a remission of only one month.

From 4 to 29 billion cells obtained from as many as ten resected ribs were infused. On the whole, as shown by the figures for the survival time (Table IV), the results were disappointing. There was no evidence of a take of the infused marrow. No clear demonstration of the presence of donor red cells (using agglutinating serums to detect C positive cells in C negative recipients of C positive marrow) was possible at any time after the marrow was injected. Likewise unsuccessful were attempts to demonstrate by methods described elsewhere [*12*] transfer of antibody producing cells from donors to recipients, as manifested by measurable increases in circulating tetanus antitoxin in the recipients. There were, however, temporary clinical improvements and, in several patients, remissions lasting from seven to ten months. (Table IV.) In every instance when radiation was repeated, the patient did not survive for more than four weeks.

The moderate amounts of radiation and steroids which were given to the patients might have been responsible for inadequate suppression of the immune mechanisms. However, in one patient (Case 11) who was admitted with a moderately severe hypokalemic alkalosis, induced by the administration of fairly large doses of ACTH and steroids, antibody formation was not decreased; another patient (Case 7) who received a minimal dose of 500 r behaved similarly. Radiation apparently eradicated the leukemic process in the peripheral blood and bone marrow in four (Cases 7, 8 and 14) of eleven trials (excluding those who died within forty-eight hours after radiation).

The dose of the treatment varied from 50 to 600 r. There was a progressive increase in the radiation dosage from the initial patient, aimed at reaching an optimal dosage which would produce the desired results. With increasing dosage, serious side effects appeared. The immediate symptoms were nausea, vomiting, diarrhea, tachycardia and oliguria. Chlorpromazine, Kaopectate® and intravenous fluids were administered to control these complications. In one patient (Case 7), who was in relapse at the time of the procedure, nitrogen retention, hyperuricemia and acidosis developed. Recovery was complete within a week; at the time of death, nine months later, the kidneys showed no evidence of radiation nephritis [*13*]. The abnormalities seen in the blood and urine constituents (Fig. 3) were consistent with acute renal failure [*14*], most probably secondary to dehydration and renal tubular obstruction by uric acid crystals. Renal complications resulting from excessive formation and excretion of uric acid in leukemic patients receiving chemotherapy or radiation have been noted [*15*]. Subsequently, this renal complication was avoided by administering alkalis and keeping the urine pH above 7.5, starting two days before radiation and continuing for the period of hyperuricaciduria. Only patients in relapse (in contrast to those in remission) showed excessive excretion of uric acid after radiation.

In four patients (five trials) hematological side effects developed during the first four weeks of radiation. The maximum depressive effect on the bone marrow appeared between the second and third week. The reticulocyte count was first to be reduced (second to fifth day), in three patients, followed closely by the leukocyte count. Platelets were the last to fall (five to eight days). Recovery of bone marrow from radiation was observed in three patients. The sequence of recovery of the blood elements after radiation was: reticulocyte count (fifteen to thirty-eight days), platelets (twenty-two to thirty-one days) and white cell count (thirty-two to thirty-eight days). In one patient the platelets reappeared in the peripheral blood before the reticulocytes. After the twelve trials of total body radiation in the nine leukemic patients, two patients died of infection (moniliasis) and two of hemorrhage (rupture of ovarian cyst, cerebral vascular hemorrhage) as a result of radiation pancytopenia. In the remainder of this group, death, when it occurred, was attributed to the leukemic process. In the

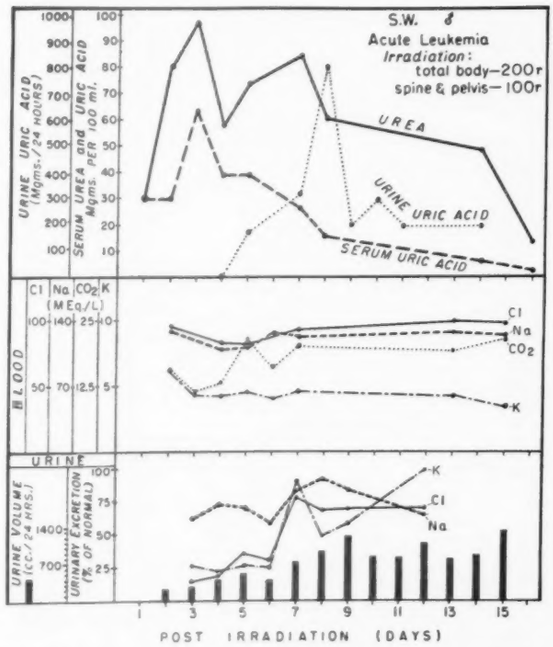


FIG. 3. Fluctuations in the blood and urine findings of a patient with acute leukemia after 300 r total body radiation.

period after radiation we attempted isolation of the patient to avoid external infection. In some patients intermittent use of antibiotics and preliminary sterilization of the gastrointestinal tract with neomycin was tried. Those in whom fungal infection developed as a result of the radiation syndrome or the leukemia *per se* were difficult to treat and succumbed to the infection, despite use of the new antifungal agents. It is difficult to assess the role that steroids played in enhancing this type of infection.

An example of the course of a leukemic patient after radiation and injection of marrow follows:

CASE 7. S. W., a four year old white boy, was admitted to Jefferson Hospital on October 28, 1957. A diagnosis of acute lymphoblastic leukemia had been made in August 1957 after a right hemiplegia developed. Subsequently, he was treated with ACTH and corticosteroids and there was partial improvement. On October 28, 1957, he was given 200 r total body radiation. He had a "cushingoid" appearance, a rectal temperature of 105°F. and a peripheral pulse of 220 per minute. He vomited intermittently and had liquid bowel movements. One day after radiation (Fig. 4A) examination of the blood revealed a hemoglobin of 14.52 gm. per cent, erythrocytes 5.84 million per cu. mm., reticulocytes 0.6 per cent, platelets 122,000 per cu. mm., leukocytes 8,985 per cu. mm. with the following differential: granulocytes 98 per

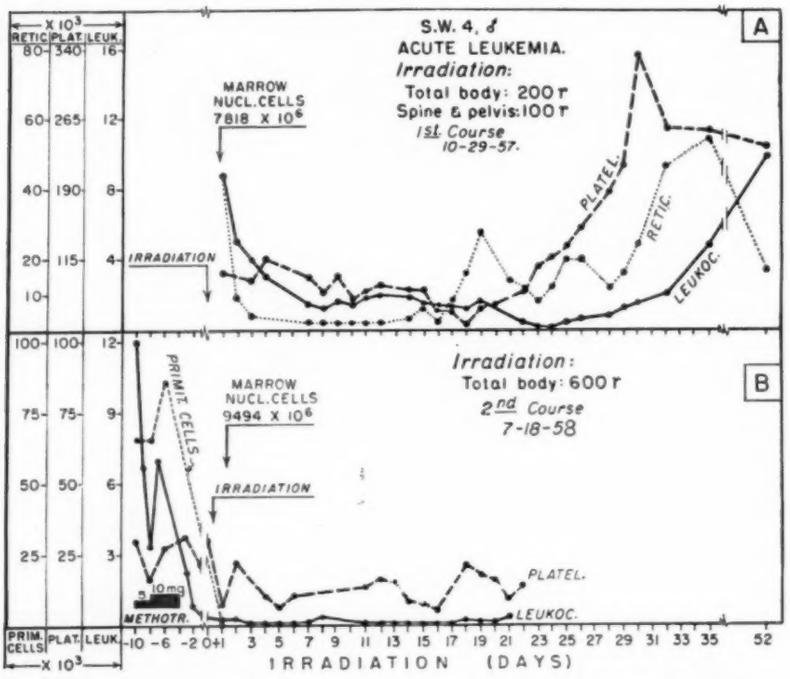


FIG. 4. Changes in the blood after total body radiation and injection of homologous marrow in a child with acute leukemia. A, total dose: 300 r. B, total dose: 600 r.

cent, lymphocytes 0.5 per cent, monocytes 1 per cent and primitive cells 0.5 per cent. Aspiration of the bone marrow disclosed a cell count of 73,500 per cu. mm., 49 per cent of which were primitive cells, many of which were semi-disintegrated. The following day the bone marrow was extremely hypocellular but primitive cells were still present. One day after radiation 7818 $\times$ 10^6 nucleated marrow cells were administered intravenously from two ribs resected from a healthy donor. The vomiting and diarrhea stopped within the next two days. There was no urinary output on the second day after radiation, and on the third day the patient had oliguria and hyperpnea. Blood chemical studies disclosed a blood urea nitrogen of 80 mg. per cent and hyperuricemia with moderate acidosis; all values returned to normal within two weeks. The blood chemical studies and the urinary excretion of electrolytes and uric acid are plotted in Figure 3. As shown in Figure 4A, there was a decrease in all the formed elements for about three weeks, after which first the reticulocytes, second the platelets and last the leukocytes began to rise. At the time of discharge the peripheral blood was essentially normal. Examination of the bone marrow disclosed 8 per cent primitive cells but it was otherwise within normal limits. The patient was then in a complete clinical remission.

The child suffered a relapse about the end of February 1958 and from March to July was given antimetabolites, which induced a partial remission. When admitted in July 1958 he looked cushingoid, had a pulse of 170 per minute and a temperature of 103°F. There was only slight enlargement of the lymph nodes. The hemoglobin was 13.7 gm. per cent, red cells 4.55 million per cu. mm., platelets 48,000 per cu. mm., reticulocytes 0.1 per cent, leukocytes 25.3 thousands per cu. mm., with the following differential: segmented neutrophils 34 per cent, metamylocytes 2 per cent, lymphocytes 4 per cent, monocytes 1 per cent and primitive cells 59 per cent. The patient's urine was kept alkaline by the intravenous infusions of $\frac{1}{6}$ molar lactate solution, and abundant fluids orally. On July 17, 1958, he received 600 r total body radiation. A suspension of marrow obtained from ribs excised from a volunteer donor was given the following day (9,494 $\times$ 10^6 nucleated cells in 300 ml. of fluid). The immediate period after infusion was relatively uneventful, except for vomiting a necrotic fragment of esophageal mucosa infested with Candida albicans. However, one week later diarrhea, abdominal distention and tachypnea appeared, and became aggravated by the third week; death occurred twenty-four days after radiation. Although there was severe acidosis (CO_2 combining power 4.8 mEq. per L.) there was no elevation of blood urea or uric acid and the serum calcium and phosphorus remained normal. The changes were consistent with a severe infection (bacterial alternating with fungal) secondary to the marked leukopenia. (Fig. 4B.) During this second treatment metabolic derangements occurred

only two days before his death and the patient maintained a normal urine output throughout this period. Autopsy revealed hypoplasia of bone marrow with foci of leukemic infiltration. There was no evidence of leukemia in the other organs. The lymphoid tissue in the lymph nodes, spleen and intestinal tract was atrophied. Mycotic (C. albicans) abscesses of the kidneys and spleen and mycotic ulcers of the cecum and stomach were evident. There was evidence of hemorrhages in the adrenal cortex and generalized petechiae and purpura.

Comment: The leukemic process was fairly well eliminated after the first course of total body radiation (200 r). Whether or not the hematological improvement that followed was due to regeneration of the patient's own marrow or induced by the infused bone marrow is difficult to say. However, the delay in the rise of the peripheral blood elements as compared to the pattern observed in experimental animals suggests that recovery was the result of regeneration of the patient's own marrow, especially since donor cells were not detected at any time following infusion of the marrow suspension.

After the second course of radiation there was striking depletion of the bone marrow as indicated by the blood elements. (Fig. 4B.) Despite the higher dosage (600 r) there was no take of the injected marrow as evidenced by either detection of donor red cells in the patient or the presence of antibodies against a foreign antigen with which the donor was challenged [*12*]. The amounts of radiation given did not seem to affect the production of antibodies. Although the patient seemed to be recovering from the hypoplastic state, he died due to an infection (bacterial and fungal), difficult to control despite such measures as efforts at isolation, sterilization of the gastrointestinal tract and the use of antimicrobial and antifungal agents.

Group 2. This group consisted of patients who received injections of bone marrow from excised ribs (without previous radiation) for aplastic anemia after being treated unsuccessfully for some time with corticosteroids and transfusions. (Table v.) Four patients (Cases 6, 12, 17 and 18) were critically ill at the time of the marrow injections. Of the remaining three, two showed clear evidence of definite although temporary improvement, clinically and hematologically. (Fig. 5.) Both patients (Cases 2 and 5) were ill for over a year without significant changes in their blood values and neither received any additional treatment. The third

TABLE V

PATIENTS WITH APLASTIC ANEMIA WHO RECEIVED INJECTIONS OF MARROW FROM EXCISED RIBS WITHOUT RADIATION

Case No.	Patient	Age (yr.) and Sex	Nucleated Marrow Cells ($\times 10^6$)	No. of Ribs	Fluid Volume (ml.)	Survival Time After Marrow Injection
2	A. S.	F., 57	691	1	24	28½ mo.
			1,458	1	20	27 mo.
			1,580	1	30	24 mo.
4	E. M.	M., 64	3,253	1	50	1 yr.
			2,756	1	52	
5	D. D.	M., 2	1,458	1	38	7½ mo.
			6,084	2	178	
6	J. W.	F., 18	2,139	1	37	10 days
12	M. L.	F., 66	3,015	1	70	4 days
17	R. G.	M., 14	1,681	1	50	7 days
18	S. W.	F., 8	6,200	2	124	2 days

NOTE: Injections administered intravenously except in patient D. D. (Case 5) in whom the route was tibial.

patient (Case 4) made no significant clinical or hematological response to two injections of marrow. (Table v.) One patient (Case 21, Table vi) who suffered from aplastic anemia complicated by septicemia, was treated with homologous marrow aspirated from multiple sites of volunteer donors, with no significant improvement. All marrow injections were well tolerated by the recipients. Illustrative case reports follow.

A. S., a fifty-seven year old white woman (Case 2, Table v), was first admitted to Jefferson Hospital in October 1955 with a history of anemia of one year's duration. For the following two years she was treated with various antianemic agents, corticosteroids, thyroid substance, testosterone and blood transfusions without significant improvement. Gradually leukopenia and thrombocytopenia developed. In June 1957 the hemoglobin was 4.06 gm. per cent; red cells 1.54 million per cu. mm.; reticulocytes 0.1 per cent; platelets 107,000 per cu. mm.; leukocytes 2,525 per cu. mm. with a differential of segmented neutrophils 50 per cent; lymphocytes 43 per cent; and monocytes 7 per cent. Three bone marrow aspirations were performed and in all the marrow was hypoplastic. An infusion of a suspension of 618 million marrow cells obtained from an excised rib was given in June 1957. As shown in Figure 5A there was a prompt increase in the platelet and leukocyte counts starting on the second day after infusion and reaching a peak at about the ninth day, after which the values gradually returned to the previous level. There was no improvement in the reticulocyte and hemoglobin values. On subsequent occasions two other infusions of marrow from excised ribs were given, but there was no significant clinical or hematological improvement. Since the last infusion

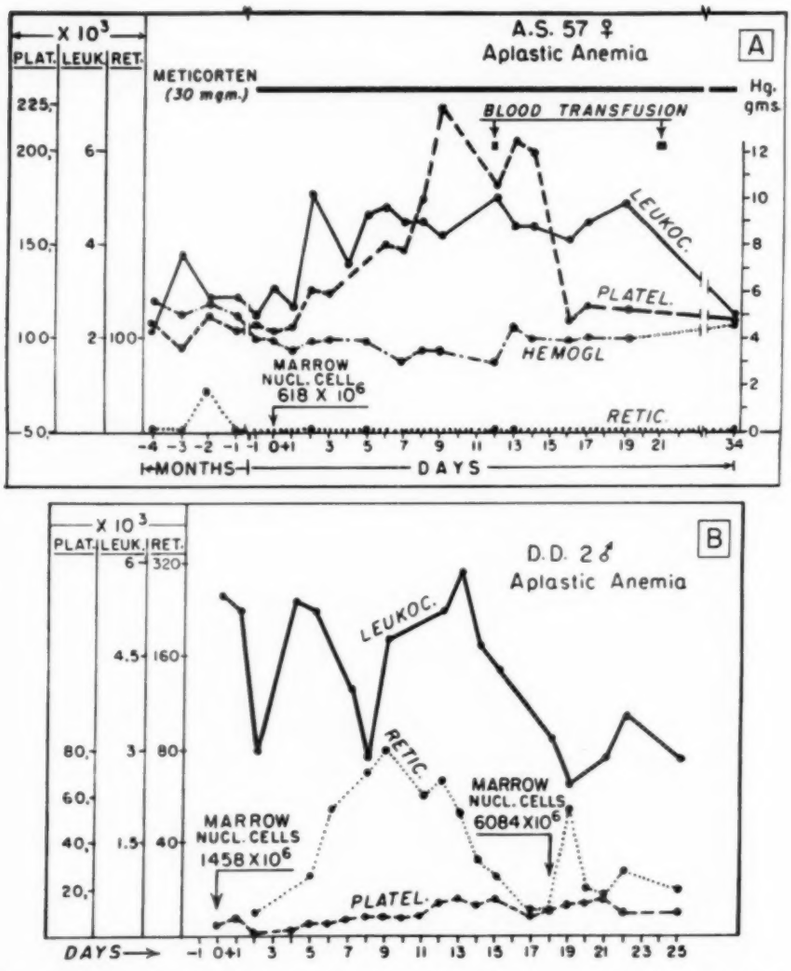


FIG. 5. Changes in the blood of two patients with aplastic anemia following injection of marrow obtained from excised ribs.

TABLE VI

PATIENTS WHO RECEIVED INTRAVENOUS INFUSION OF BONE MARROW ASPIRATED FROM BONES OF VOLUNTEER (BLOOD GROUP COMPATIBLE) DONORS (HOMOLOGOUS MARROW) OR FROM THE PATIENTS THEMSELVES (AUTOLOGOUS) BEFORE SUPPRESSIVE THERAPY (HN_2)

Case No.	Patient	Diagnosis	Age (yr.) and Sex	Nucleated Cells ($\times 10^6$)	Source of Marrow	Fluid Volume (ml.)	Therapy Before Marrow Injection	Survival Time
20	J. P.	Malignant lymphoma	M., 56	2,936	Homologous	58	Corticosteroids	7½ mo.*
21	B. Y.	Aplastic anemia	F., 16	3,447	Homologous	84	Corticosteroids	8 days
22	M. G.	Reticulum cell sarcoma	F., 21	4,905*	Autologous	292	HN_2, 100 mg.	6 mo.†
23	D. D.	Mycosis fungoides	M., 36	2,283‡	Autologous	338	HN_2, 32 mg.	2 mo.†

* Marrow preserved at −65°C. for thirteen days before being returned to the patient.
† Still living (February 1960).
‡ Patient's own marrow returned to him three hours after the nitrogen mustard was administered.

TABLE VII

PATIENTS WITH ACUTE LEUKEMIA IN AN APLASTIC PHASE (INDUCED BY ANTIMETABOLITES) WHO RECEIVED INTRAVENOUS INJECTIONS OF BONE MARROW FROM EXCISED RIBS WITHOUT PREVIOUS RADIATION

Case No.	Patient	Age (yr.) and Sex	Nucleated Marrow Cells ($\times 10^6$)	No. of Ribs	Fluid Volume (ml.)	Preliminary Therapy	Survival Time After Marrow Injection
1	M. H.	F., 52	1,377	1	18	6-Mercaptopurine	27 days
16	O. N.	M., 38	19,850	4	238	Amethopterin, 6-Mercaptopurine	6 mo.
19	H. F.	F., 57	2,328	1	35	6-Mercaptopurine	5 mo.

of marrow, given twenty-four months ago, it has been necessary to give the patient 2 units of packed red cells every three weeks to maintain a hemoglobin level of 6 to 7 gm. per cent.

D. D., a two year old white boy (Case 5, Table V), was admitted with the diagnosis of aplastic anemia. Soon after birth he was found to have thrombocytopenia. One year later he became anemic. Since that time he has been admitted to other hospitals for the management of infection, epistaxis, bleeding of the gums, hematuria and melena. Treatment has consisted of blood transfusions, corticosteroids and antibiotics. When admitted to Jefferson Hospital he had a cushingoid appearance, petechiae and bruises over the entire body, and a slightly enlarged liver. The rest of the physical examination was noncontributory. The hemoglobin was 6.4 gm. per cent, red cells 2.23 million per cu. mm., platelets 5,000 per cu. mm., leukocytes 5,500 per cu. mm. with a differential of 13 per cent segmented neutrophils, 68 per cent lymphocytes, and 19 per cent monocytes. Tibial bone marrow aspiration disclosed severe hypoplasia. The child was given, at an interval of eighteen days, infusions intratibially (peripheral veins unavailable) of $1,458 \times 10^6$ nucleated cells and $6,084 \times 10^6$ nucleated cells obtained from marrow of excised ribs. Therapy with prednisolone, blood transfusions and antibiotics was continued as before the infusions. There was a significant increase in the reticulocyte count starting four days after the first infusion, reaching a peak on the sixth day and lasting fifteen days. (Fig. 5B.) Clinically, there was rapid fading of the petechiae which, however, started to recur after two weeks. Gastrointestinal bleeding, which had continued for three days, ceased after the second infusion of marrow. The child died about seven months later of intracerebral hemorrhage.

Comment: The time pattern of increase in the leukocyte, platelet and reticulocyte counts in these two patients closely resembles that seen after the transplantation of homologous tissues. Homologous skin transplants, soon after they are grafted, appear to take, only to be rejected by the recipient between eight and twelve days after the transplant. It seems that in these two patients a temporary take of the donor marrow took place. During this interval reticulocytes, platelets and leukocytes were produced. At about the ninth day the marrow was rejected and the production of new cells was back at its former level. It is not possible to be sure, however, that the increases did represent donor cells; the injected donor marrow might conceivably have stimulated (humoral effect) a greater activity of the recipient's own marrow cells. The coincidence of the duration of this "temporary take" with that of the survival of other homologous tissues (e.g., skin) makes the first explanation seem more plausible.

Group 3. This group consists of patients who received bone marrow during the drug-induced hypoplastic or aplastic phase of an acute leukemia or malignant lymphoma. Three patients were given marrow from excised ribs after therapy with variable doses of antifolics or antipurines. (Table VII.) At the time all these patients were acutely ill (high fever, mouth ulcers, generalized bleeding from the mucous membranes) and had to be given supportive therapy with transfusions for several days after cessation of the antimetabolite therapy. In two patients (Cases 1 and 16) the bleeding ceased and the hemorrhages began to lessen within twenty-four hours after the infusion of the marrow, before any change in the platelets was discernible. All three patients entered a period of clinical and hematologic remission; in two it lasted several

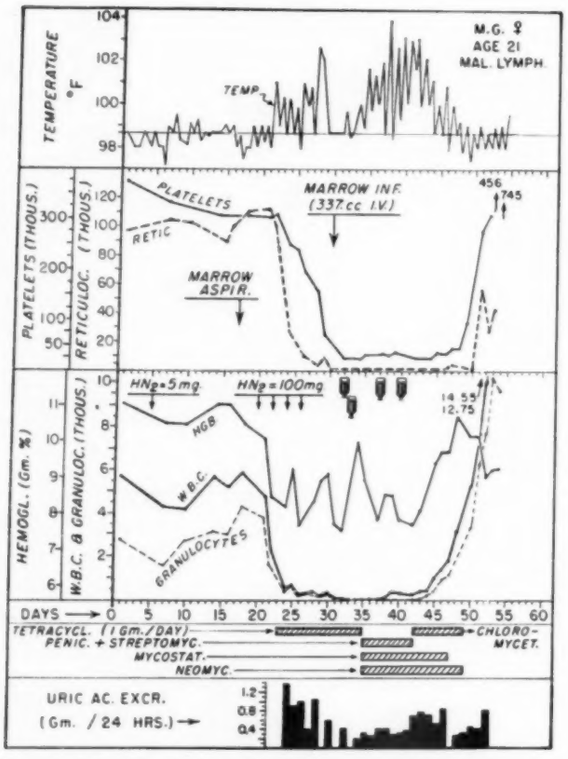


FIG. 6. Case 22 (Table VI). Changes in the blood, urine and body temperature of patient who was given nitrogen mustard followed by autologous marrow.

months but in the other (Case 1) a fall caused her death on the twenty-seventh day after the infusion of marrow. Immunological and red cell type studies failed, however, to provide any support for a take of the donor marrow. Nevertheless, it was difficult to escape the belief that the antihemorrhagic and antipyretic effect, lasting for about four days after transplantation, may have reflected a transient functioning of the transplanted megakaryocytes and leukocyte precursors. Previous transfusions of fresh blood had had no significant hemostatic effects.

Two patients (Cases 22 and 23), (Table VI) were given their own marrow following administration of nitrogen mustard. One patient (Case 23) had mycosis fungoides with severe pancytopenia, but an active hyperplastic marrow; he made a fair response to the procedure but suffered a relapse one month later and underwent a splenectomy.

A good example of the possible use of autologous marrow to extend the period of the remission after suppressive therapy of the malignant lymphomas follows:

M. G., a twenty-one year old woman (Case 22, Table VI), was admitted to the hospital on July 20, 1959, with the complaint of swelling of the neck of five months' duration. Except for a 10 pound weight loss and some fatigue, she had no other complaints. There was moderate enlargement of lymph nodes throughout the body, particularly the inguinal and pelvic areas. The spleen was just palpable. Hemoglobin was 11.1 gm. per cent, red cells 3.7 million per cu. mm., platelets 374,000 per cu. mm., leukocytes 5,700 per cu. mm. with a differential of 50 per cent segmented neutrophils, 3 per cent eosinophils, 39 per cent lymphocytes and 8 per cent monocytes. The bone marrow was normal. Roentgenographic study of the chest revealed slight hilar and tracheal lymphadenopathy. A biopsy specimen of a lymph node disclosed a reticulum cell sarcoma.

On August 5, 1959, under general anesthesia, about 350 cc. of the patient's bone marrow was aspirated from multiple sites in the sternum, iliac crests and posterior inferior iliac spines. The marrow was aspirated into a phosphate buffer sequestrene solution, and glycerol added to a final concentration of 15 per cent. It was distributed into several tubes and frozen slowly by a technic described elsewhere [*16*]. On August 9 the patient was given 100 mg. of nitrogen mustard in four divided doses (25 mg. each) on alternate days. A bone marrow aspiration obtained on August 17 disclosed an almost complete aplasia. Two days later (August 19) she was given intravenously the thawed out marrow ($4{,}905 \times 10^6$ nucleated cells) which had been kept at $-65°$C. since it was collected. During the next thirty-six hours she passed 13.6 gm. of hemoglobin in the urine, a result of the hemolysis of her own glycerolized red cells in the marrow suspension. Most of the changes before and after HN_2 was given, as well as the supportive therapy with transfusions and antibiotics, are shown in Figure 6.

About ten days after the last dose of nitrogen mustard was administered, the leukocyte count was virtually zero and the patient was quite ill. There were ulcerations in the tongue, oral and pharyngeal mucosa and reddening of the tympanic membranes. The hair of the scalp began to fall out and within two weeks most of it had disappeared. The urinary output of uric acid, which was about three times normal when treatment was begun, was markedly reduced during the leukopenic period and began to return to the normal level about twelve days after the infusion of marrow. From this point on the size of the lymph nodes continued to regress rapidly and the patient began to improve. Six months after the treatment her general condition remains excellent. Her hemoglobin is 12.5 gm. per cent, red cells 4.3 million per cu. mm., platelets 315,000 per cu. mm., leukocytes 5,500 per cu. mm. with a normal differential. Most of the scalp hair has grown back. No enlargement of the

lymph nodes has been evident since discharge from the hospital.

Comment: In view of the degree of malignancy of reticulum cell sarcomas and their notoriously poor response to suppressive therapy, the result in this patient is distinctly better than that obtained in most patients with this type of tumor. Did the injection of autologous marrow help to accelerate recovery of the patient's own marrow? Did it supply proliferative marrow cells at a time when most of the patient's marrow was destroyed? Or did it simply provide the patient with functioning marrow tissue to overcome partially the septic and hemorrhagic state and help the patient survive until the time when her own remnants of marrow would recover and begin to function? Answers to these questions can seldom be given on the basis of clinical experience. Experimental evidence [*17*] seems to indicate that autologous marrow does provide some protection against the marrow depression which follows exposure to radiation.

COMMENTS

Although we have, like others [*18,19*] failed to obtain a successful transplant of human marrow, certain useful clinical facts have been learned as a result of these attempts. Avenues of approach for the procurement of marrow from living and non-living sources have been surveyed, and methods for processing and administering such marrow to human subjects have been worked out in practice. Preservation of marrow by freezing may well help in extending the use of autologous marrow as a protection from the hypoplastic or aplastic effect of certain physical and chemical agents. The temporary take which probably occurs after homologous marrow transplantation may serve to protect the recipient, for a short period, from the septic and hemorrhagic complications of acute aplastic states. This latter possibility still requires further investigation; if shown to exist, it may well constitute the only justification for injections of homologous marrow at the present time.

The reasons for failure to obtain successful transplants in our trials are apparent. The conditions shown to have led to a successful transplant in experimental animals exposed to lethal doses of total body radiation have not as yet been approached in man. The technic and accurate dosimetry of total body radiation in human beings are still in a tentative stage.

The early expectations from the work with transplantable murine leukemias [*2,3*] that radiation may destroy the leukemic tissue have not been supported by recent studies [*20*]. The dose of total body radiation necessary to sterilize leukemic cells *in vivo* has varied from 2,000 to 2,500 r in certain forms of leukemia to over 5,000 r in others [*20*]. These doses are considerably beyond the range of the protection afforded by injections of marrow.

Transplantation of bone marrow has to be considered on the same basis as a tissue or organ transplant. Much of what was learned during the past fifty years regarding transplantation of skin seems to apply equally well to transplantation of marrow. Unlike transfusion of blood, which does not require cells of proliferative capacity, a successful transplant of marrow may well depend to a large extent on the number of such cells. There is as yet no general agreement as to which cell accounts for the radiation protective effect of injections of marrow.

The laws of acceptance of tissue grafts must also be held valid for marrow grafts. Nonisologous tissue is always rejected and the time of its rejection is closely related to the genetic composition or compatibility between donor and recipient. Cellular, not humoral immunological mechanisms are involved in the rejection of grafts, so that detection of incompatibility previous to transplantation may not be possible. Moreover, certain practices such as the pretesting of potential donors by grafting their skin in the tentative recipient are unwise, since this might well sensitize the recipient and accelerate rejection of any future grafts from that donor. In this connection, transfusions of blood given to the recipient before attempts at transplantation of marrow may well act as sensitizing agents, particularly if the blood comes from the prospective donors of marrow. These and other considerations illustrate the complexity of the problem and the necessity for further work on the mechanisms of tolerance to homologous tissue grafts, the nature of the rejection phenomenon, and methods for counteracting it during the initial stages of the grafting. Moreover, once a successful graft of homologous marrow is accomplished, the onset of "secondary (or homologous) disease" must be anticipated.

Lessons learned in the control of the "host reaction" may well help later in the management of this complication.

SUMMARY

1. Twenty-six attempts at transplantation of human bone marrow obtained from excised bones were carried out in nineteen patients with acute leukemia and other marrow depletion syndromes with or without preliminary treatment with total body radiation. There was no evidence of a permanent "take" after any of the attempts, although signs of transient functioning of the marrow appeared in three instances.

2. Four additional patients received marrow *aspirated* from living donors (two homologous and two autologous). One patient with reticulum cell sarcoma underwent a relatively long remission after receiving 100 mg. of nitrogen mustard, followed by injection of her own preserved marrow.

3. The course of the patients with acute leukemia did not in general seem to be altered significantly by radiation and injections of marrow. The explored sources of procurement and methods of processing and administering marrow from excised bones may provide a basis for the utilization of human marrow in future attempts to transplant bone marrow.

REFERENCES

1. Congdon, C. C., Uphoff, D. and Lorenz, E. Modification of acute irradiation injury in mice and guinea pigs by injections of bone marrow: a histopathologic study. *J. Nat. Cancer Inst.*, 13: 73, 1952.
2. Barnes, D. W. H., Corp, M. J., Loutit, J. F. and Neal, F. E. Treatment of murine leukaemia with x-rays and homologous bone marrow. I. *Brit. M. J.*, 2: 626, 1956.
3. Barnes, D. W. H. and Loutit, J. F. Treatment of murine leukaemia with x-rays and homologous bone marrow. II. *Brit. J. Haemat.*, 3: 241, 1957.
4. Tocantins, L. M. Potential clinical application of transplantation of preserved bone marrow. In: Proceedings of the Sixth Congress of the International Society Hematology, p. 401. New York, 1957. Grune & Stratton.
5. Congdon, C. C. Recovery from irradiation injury with special consideration of the use of bone marrow transplantation. In: Progress in Hematology, vol. 2, p. 21. New York, 1959. Grune & Stratton.
6. Transplantation of bone marrow. Proceedings of the Blood Club, 1957 Meeting, Atlantic City. *Blood*, 13: 266, 1958.
7*a*. Summary of conferences on radiation protection and bone marrow transplantation. *Blood*, 13: 288, 1096, 1958; *Blood*, 14: 602, 1250, 1959.
7*b*. Merrill, J. P. Transplantation of normal tissues. *Physiol. Rev.*, 39: 860, 1959.
8. Urso, P. and Congdon, C. C. The effect of the amount of isologous bone marrow injected on the recovery of hematopoietic organs, survival and body weight after lethal irradiation injury in mice. *Blood*, 12: 251, 1957.
9. Weston, J. K., Maxwell, R. E., Lee, M., Finzel, J. and Fisken, R. A. Curative effect of rat bone marrow transfusions in aplastic (severe hypoplastic) anemia in rats induced by myleran, a radiomimetic chemical. *Fed. Proc.*, 16: 377, 1957.
10. Goodman, J. W. and Congdon, C. C. The killing effect of blood—bone marrow mixtures given to irradiated mice. To be presented at meeting of Radiation Research Society, San Francisco, May 8–11, 1960.
11. Billen, D. Recovery of lethally irradiated mice by treatment with bone marrow cells maintained in vitro. *Nature*, 179: 574, 1957.
12. Stoloff, I. L., Haurani, F. I., Repplinger, E. F. and Havens, W. P. Effects of total body irradiation on the production of antibody in man. *New England J. Med.*, 260: 1258, 1959.
13. Luxton, R. W. Radiation nephritis. *Quart. J. Med.*, 22: 215, 1953.
14. Swann, R. C. and Merrill, J. P. Clinical course of acute renal failure. *Medicine*, 32: 215, 1953.
15. Sandberg, A. A., Cartwright, G. E. and Wintrobe, M. M. Studies on leukemia. I. Uric acid excretion. *Blood*, 11: 154, 1956.
16. Schwartz, I. R., Repplinger, E. F., Congdon, C. C. and Tocantins, L. M. Transplantation of bone marrow after preservation at −70°C. *J. Appl. Physiol.*, 11: 22, 1957.
17. Mannick, J. A., Lochte, H. L., Jr., Ashley, C. A., Thomas, E. D. and Ferrebee, J. W. Autografts of bone marrow in dogs after lethal total body irradiation. *Blood*, 15: 255, 1960.
18. Mathé, G., Bernard, J., Schwarzenberg, L., Larrieu, M. J., Lalanne, C. M., Dutreix, A., Denoix, P. F., Surmont, J., Schwarzmann, V. and Ceora, B. Attempts at treatment of leukemic subjects during remission by total body irradiation followed by transfusion of homologous marrow. *Rev. franç. Etudes Clin. Biol.*, 4: 675, 1959.
19. Thomas, E. D., Lochte, H. L. and Ferrebee, J. W. Irradiation of the entire body and marrow transplantation: some observations and comments. *Blood*, 14: 1, 1959.
20. Burchenal, J. H., Hemphill, S., Holmeerg, E. and Weigand, L. Sterilization effects in vivo of large single doses of x-ray on a spectrum of mouse leukemias. (Abstract.) *Proc. Am. A. Cancer Res.*, 2: 284, 1958.

End of Symposium

Clinical Studies

Primary Hemorrhagic Thrombocythemia*

FARUK L. OZER, M.D., WAYNE E. TRUAX, M.D., DAVID C. MIESCH, M.D.† *and* WILLIAM C. LEVIN, M.D.

Galveston, Texas

SINCE the original report by di Guglielmo [*1*] in 1920 a controversial syndrome has appeared in the literature under a variety of names: "piastrinemia" [*1*], "hemorrhagic thrombocythemia" [*5*], "primary thrombocythaemia" [*6*], "megakaryocytic leukemia [*13, 27*], "hyperthrombocytic myelosis" [*18*], "essential thrombocythemia" [*10,15*], "primary hemorrhagic thrombocythemia" [*24*], "thrombocythemia" [*22*], and "essential thrombophilia" [*7*]. As a parallel to "polycythemia vera," the term "thrombocythemia vera" appears suitable; however, for fear of further confusion, "primary hemorrhagic thrombocythemia" is preferred.

The purpose of this paper is to report our experience with six or possibly seven cases of this syndrome, to review the available world literature on this subject, and to propose the diagnostic criteria for this syndrome.

ANALYSIS OF CASES

The youngest patient was twelve years of age, the oldest sixty. With one exception, the patients ranged in age between forty-eight and sixty years. There were four women and two men. The family histories were negative for hematologic disorders. Referral diagnoses included anemia, epilepsy and brain tumor.

The symptoms were mainly related to thrombohemorrhagic phenomena, anemia and easy bruising or other purpuric manifestations. Spontaneous, prolonged epistaxis was common, as was gingival bleeding, particularly after tooth extractions. There was one instance of hemoptysis and one of angina pectoris among the patients studied. All but one had experienced gastrointestinal bleeding but only one patient gave a history compatible with peptic ulcer. Pressure symptoms due to hepatosplenomegaly were prominent in two patients. One patient had hematuria, one had vaginal spotting ten years after menopause, and one patient had priapism. One patient experienced epileptic seizures late in life, and a few years preceding the diagnosis of primary hemorrhagic thrombocythemia. One patient was hemiplegic when first seen and another described symptoms suggestive of carotid artery insufficiency. Three patients reported paraesthesias of the extremities; in one of these this symptom was so severe that it led her to seek medical attention. One patient had symptoms of sudden arterial occlusion of one leg. There was severe postoperative bleeding from operative sites in one patient.

The signs elicited on physical examination corresponded again to thrombohemorrhagic phenomena and anemia, when present. Hemorrhages varying from petechiae to large ecchymoses were present in all. Lymphadenopathy was absent in all the patients. In three patients there was oozing of blood from the gums. Mild to moderate splenomegaly was observed in all patients. Slight to moderate hepatomegaly was present in all but two patients. One patient had Jacksonian epilepsy while under observation and another patient was hemiplegic, as already noted. The blood pressure was moderately elevated in two patients.

LABORATORY FINDINGS

The initial blood counts are shown in Table 1. In one patient (V. L.) the hemoglobin and red blood cell levels were normal, in two (F. A. and R. E.) these values reflected slight erythremia. Three patients (V. L., L. E. and B. G.) were markedly anemic. In two patients (L. E. and F. A.) a few immature leukocytes were seen. Slight eosinophilia was present in three (V. L.,

* From the Department of Internal Medicine, Hematology Research Laboratory, University of Texas Medical Branch, Galveston, Texas.

† Present address: Paris, Texas.

TABLE 1

PERIPHERAL BLOOD COUNTS OF SIX PATIENTS AT THE INITIAL EXAMINATIONS

Patient, Age (yr.) and Sex	Hemoglobin (gm. %)	Red Blood Cells (million/cu. mm.)	Hematocrit (%)	White Blood Count (per cu. mm.)	Polymorphonuclears (%)	Stab Forms (%)	Myelocytes (%)	Metamyelocytes (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)	Lymphocytes (%)	Platelets (cu. mm.)	Reticulocytes (%)
V. L., 12, F	12.4	4.4	....	19,700	63	..	1	..	5	11	4	18	5,528,000	1
B. G., 60, M	4.7	2.93	....	11,600	75	..	..	..	1	5	9	10	1,582,200	5
L. E., 53, F	6.3	2.95	....	26,750	76	3	4	3	2	4	9	3	3,447,000	3.6
F. A., 52, M	16.4	5.74	54	11,600	60	6	1	1	4	2	13	13	1,835,000	0.4
R. E., 58, F	16.8	6.20	54	20,150	77	..	..	..	4	2	6	11	6,940,000	1.6
H. H., 49, F	5.0	2.19	20.0	20,700	79	..	..	..	1	2	2	6	2,417,760	2.6

F. A. and R. E.), slight to moderate basophilia in three (V. L., L. E. and B. G.).

Platelet levels varied between 1,582,000 and 6,940,000 per cu. mm. (Normal values in our laboratory range between 250,000 and 800,000 per cu. mm.) The morphology of the platelets also was abnormal and varied from case to case. The platelets were either small or large, and many bizarre forms were seen. Fragments of megakaryocytes were occasionally observed. The peripheral blood picture remained essentially the same during the period of observations. Platelet levels fluctuated from day to day, usually remaining above 1,000,000 per cu. mm.

Bone marrow examinations revealed hyperplasia of all the marrow elements. In each case eosinophils and basophils were slightly to moderately increased. In one patient the marrow was megaloblastic. The most striking finding was the pronounced increase in the number of the megakaryocytes in every case (Fig. 1), and numerous megakaryoblasts were invariably present. Large masses of platelets could be seen in almost every field examined. In one patient (R. E.) one marrow aspirate consisted almost solely of platelets. (Fig. 2.)

Detailed coagulation studies revealed an abnormality in only one patient (V. L.)

In the presence of anemia, the stools were always guaiac-positive; however, a definite source of bleeding could never be demonstrated despite extensive x-ray and endoscopic examinations. Air studies and bilateral carotid arteriograms in the one patient admitted because of epilepsy gave negative results.

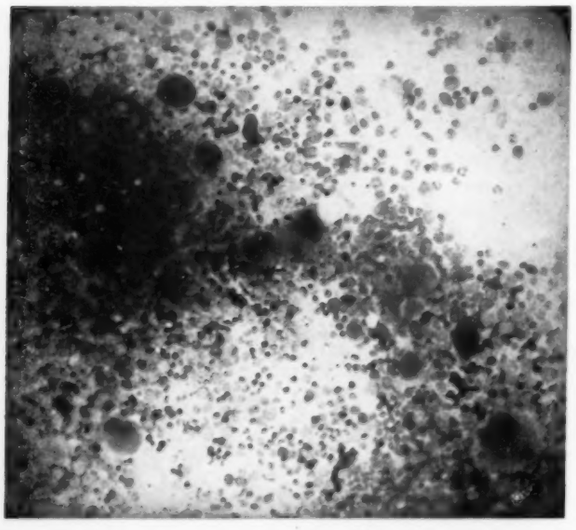


FIG. 1. Bone marrow aspiration film. Note increased number of megakaryocytes (lower magnification).

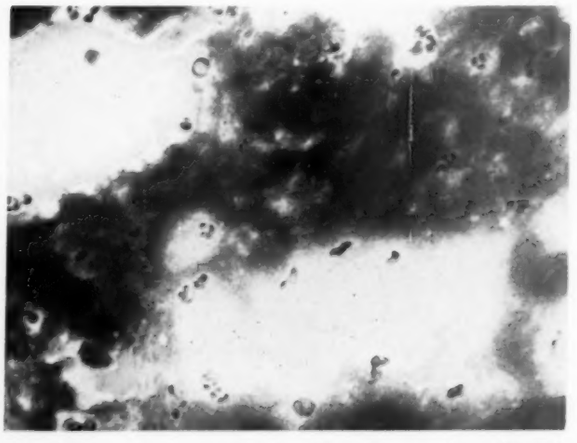


FIG. 2. Bone marrow aspiration film of one patient (R. E.) which consists almost solely of platelets.

In the following studies results were either negative or within normal limits: Roentgenograms of the skull, intravenous pyelogram, cholecystogram, liver function studies, uric acid levels (serum and urine), non-protein nitrogen, fasting blood sugar, L.E. phenomenon, serum electrophoresis and serum electrolytes. Electrocardiograms were normal in all patients. An electroencephalogram was normal in one patient with epilepsy. Biopsies of the liver, muscle and subcutaneous nodules were negative, and without complication.

SUBSEQUENT COURSES

In the first patient (V. L.) in this series acute myeloblastic leukemia developed three years after she was first seen here; she died elsewhere. The second patient (B. G.) experienced arterial occlusion of one lower extremity over a period of two to three days while thrombocythemia was still present. With paravertebral sympathetic block he seemed to be improved, and an emergency sympathectomy was performed without complications. Three months later a transmetatarsal amputation was performed. Pathologic examination of the amputated specimen revealed generalized arteriosclerosis.

The last patient (H. H.) had a complicated course. Pneumonia and thrombophlebitis developed early in her hospital stay; both responded to treatment. The urine volume was high, usually being over 8,000 cc. per twenty-four hours. Unfortunately, because of disturbed mental status, this problem could not be adequately studied. Later symptoms of carotid artery insufficiency developed, the patient lapsed into coma and died. Ten days prior to her death she was given 10 mg. of nitrogen mustard. At necropsy there was no evidence of leukemia and the cause of death was brain hemorrhage. The other patients are being followed up.

TREATMENT

Radioactive phosphorus was administered to six patients and nitrogen mustard to one patient. P^{32} was administered intravenously in divided doses over a period of one to three weeks. The dose of each course was between 5 and 9.5 mc. Hematologic remissions usually occurred about three weeks after initiation of treatment. Clinical remissions appeared even sooner. Except for easy bruising, the bleeding tendencies completely abated. The patient (R. E.) who had angina pectoris and symptoms of carotid artery insufficiency was symptom-free three months later. In another patient (F. A.) epileptic seizures occurred less frequently following therapy. The size of the spleen and the liver receded or returned to normal in all patients.

The hematologic and clinical remissions lasted from five to thirteen months. In only one patient (V. L.) did major bleeding episodes recur, responding once again to treatment. In this patient acute myeloblastic leukemia developed later after the fourth course of treatment with P^{32}.

The two patients who received two courses of therapy with P^{32} were in remission when they were last observed. One patient (H. H.) in this series received a total of 20 mg. of nitrogen mustard because of the acuteness of her condition; this produced no amelioration of the disease and the patient died.

REVIEW OF THE LITERATURE

In 1920 di Guglielmo [*1*] described a patient with erythroleukemia and marked increase in platelets and coined the term "piastrinemia." This case, apparently, was one of panmyelosis, not primary thrombocythemia. In 1934 Epstein and Goedel [*2*] described a patient with slight erythrocytosis, moderate leukocytosis and recurrent thrombocytosis. Later the patient had anemia due to bleeding; he died following an operation for impending gangrene of the toes. Necropsy revealed an atrophic spleen weighing 7 gm., but there was no evidence of leukemia. In 1940 Reid [*9*] reported a case of "hemorrhagic thrombocythaemia" and suggested diagnostic criteria for the disease entity. Fanger et al. [*22*] in 1954 reviewed the literature and reported three additional cases. The first patient in this series was remarkable. He had up to 14,000,000 platelets per cu. mm. with only slight bruising. The second patient died one day after admission and necropsy revealed hemorrhagic infarctions of the adrenal glands. According to these authors only these two cases could be classified as true thrombocythemias, all the other cases previously reported in the literature were interpreted to be examples of secondary thrombocytosis. In 1955 McCabe et al. [*24*] reviewed the literature and reported a case; three years later acute myelogenous leukemia developed in this patient.

In 1957 a symposium in *Le Sang* [*28–32*] included several case reports and studies on the pathogenesis of thrombocytoses. Levinson et al. [*37*] recently described a most unusual case of

TABLE II

CASES OF "PRIMARY HEMORRHAGIC THROMBOCYTHEMIA" REPORTED IN THE LITERATURE

Authors and Year	Age (yr.) and Sex	Red Blood Cells (million per cu. mm.)	Hemoglobin (%)	Hematocrit (%)	White Blood Cells (per cu. mm.)	Platelets (per cu. mm.)	Peripheral Blood Smear	Bone Marrow	Follow Up (yr.)	Remarks
di Guglielmo, 1920 [1]	?	7.5	?	.....	20,000	Marked increase	.....	Not reported	.....	Erythro-leukemia and piastrinemia
Epstein, Kretz and Goedel, 1930–1934 [2]	56,M	5.5–7.25 3.0	115 44	.....	12,500–20,000	1,200,000 – 2,200,000	Monocytosis 12–33%, eosinophilia 6–23%, left shift, normoblasts	Great excess of megakaryocytes	4	Splenic atrophy
Drake, 1936.....	65,F	4.5–5.8	70–84	.....	22,000–33,000	1,000,000 – 2,580,000	Normal differential at outset, later leukemic	Not reported	5 mo.	Probably chronic granulocytic leukemia
Uotila, 1938.....	64,M	4.8–7.28	40–113	.....	17,000–40,000	1,000,000 – 5,000,000	Eosinophilia 2–5%, basophilia 2–5%	Greatly increased megakaryocytes with young forms	2	Probably polycythemia vera
Nygaard and Brown, 1938	47,M	4.52	15.2	.....	17,000	2,480,000 – 2,450,000	Normal	Not reported	.....	Gangrene of toes, episodes of acute pancreatitis
Rowlands and Vaizey, 1938	59,M	2.8	28	.....	8,600–12,800	1,237,000 – 1,900,000	Eosinophilia 30%	Not successful	6	Steatorrhea
	40,F	5.4–7.18	94 120	.....	8,000–18,000	492,000 – 1,820,000	Occasional eosinophilia	Not reported	14 mo.	Probably polycythemia vera
Reid, 1940.....	54,F	4.58–5.05	13.6–14.9	38–51	14,000–16,000	1,730,000 – 3,800,000	Occasional myelocytes	Extraordinary increase in megakaryocytes	6 mo.	Thrombosis of deep veins
Owren, 1943.....	49,F	4.42–4.88	88–89	.....	8,000–13,000	1,180,000 – 1,440,000	Normal	Increased megakaryocytes, enormous masses of platelets	5	Peptic ulcer, no hemorrhagic tendencies
Forssell, 1947....	52,F	2.0–7.28	25–108	.....	12,000–38,000	428,000 – 1,688,000	Few immature leukocytes, Howell-Jolly bodies	Hypercellular	20	Small calcified spleen
Holst, 1948.....	45,M	3.64–5.3	89–98	.....	19,000–31,000	625,000 – 932,000	Eosinophilia, 4–8% slight left shift	Hypercellular, increased megakaryocytes	5	.....
Jasinski, 1948....	69,F	4.2	88	.....	27,000	1,780,000	Left shift, basophilia 6%, normoblasts	Hypercellular, numerous megakaryocytes	4 mo.	Spleen and liver punctures negative for leukemia
Hemmeler, 1948..	29,F	4.13	83	.....	11,600	2,600,000 – 3,300,000	Slight left shift with eosinophilia and basophilia	Extreme increase in megakaryocytes, platelet masses	15 mo.	Treated with urethane and arsenic
Moolten et al., 1949	2	.....	.....	.....	95,000	702,000	High proportion of myelocytes	Not reported	.....	Thrombosis of femoral artery, necropsy: no evidence of leukemia
	31,F	.....	.....	.....	.....	580,000	.....	Not reported	.....	Insufficient information
	2	6.47	.....	.....	.....	1,126,000	.....	Increased megakaryocytes	.....	Insufficient information
Mortensen, 1948..	64,M	2.56–9.79	45–125	.....	20,900–47,800	220,000 – 6,000,000	Slight eosinophilia	Hypercellular, eosinophilia, megakaryocytes, increased platelet masses	.....	Probably polycythemia vera
Spangberg and Zettergren, 1949	45,F	4.12	62	.....	11,600	220,000 – 1,890,000	Normal	Increased megakaryocytes, platelet masses	6 mo.	Cancer of stomach, splenectomy
Revol, 1950.....	41,M	2.95	46	.....	4,800– 6,800	1,480,000	Normal differential, platelet masses	Increased megakaryocytes	.....	Treated with x-ray before platelet count known, later splenectomy
	60,F	4.6–5.6	78–90	.....	19,000	1,800,000 – 2,800,000	Slight left shift	Hypercellular, increased megakaryocytes	3	Low fibrinogen
Stenström, 1950..	70,M	3.0	42	.....	11,000	712,000 – 1,400,000	Normal	Greatly increased megakaryocytes	.....	Necropsy: no evidence of leukemia; peptic ulcer
	71,M	.....	60	.....	6,000	900,000 – 1,400,000	Slight left shift	Greatly increased megakaryocytes, platelet masses	1	Treated with P^{32}
Söderström, 1947.	63,M	4.9–5.6	71	.....	12,000–23,400	1,710,000 – 1,200,000	Few immature white cells	Hypercellular, increased megakaryocytes	.....	Bleeding time, 16 minutes
Monto et al., 1952.	65,M	4.51	12.7	.....	38,900–42,500	906,000	Moderate left shift	Hypercellular, increased megakaryocytes, platelet masses	1 mo.	Necropsy: multiple pulmonary infarction; no leukemia
	37,M	3.7–4.2	.....	.....	6,300–22,100	10,800,000 –14,030,000	Normal	Megakaryocytes increased	4	No bleeding or thrombotic tendency
Fanger et al., 1954.	67,F	3.91–4.2	9.5–12.3	32	5,200	1,470,000	Normal differential	Great megakaryocytic hyperplasia	.....	Necropsy: hemorrhagic infarction of adrenals
	63,F	5.0	15.0	.....	17,400	.....	Granulocytosis	.....	.....	Before splenectomy, later splenectomized with resultant thrombocytosis

TABLE II (*Continued*)

CASES OF "PRIMARY HEMORRHAGIC THROMBOCYTHEMIA" REPORTED IN THE LITERATURE

Authors and Year	Age (yr.) and Sex	Red Blood Cells (million per cu. mm.)	Hemoglobin (%)	Hematocrit (%)	White Blood Cells (per cu. mm.)	Platelets (per cu. mm.)	Peripheral Blood Smear	Bone Marrow	Follow Up (yr.)	Remarks
McCabe et al., 1955	44,M	3.0–5.2	10.0–18.8	.....	5,000–314,000	6,300,000 – 40,000	Normal differential at outset; later leukemic	Megakaryocytic hyperplasia at outset, later: myelogenous leukemia	26 mo.	Transformation to myelogenous leukemia
Woodrow and Cope, 1955	54,F	4.9	58	.....	10,800	2,400,000	Normal with target cells	Hypercellular, striking increase in megakaryocytes	10 mo.	Treated with P^{32}
	46,M	.....	8.2	.....	30,000	Normal to 1,395,000	Normal	Hypercellular, greatly increased megakaryocytes	38 mo.	Duodenal ulcer, atrophic spleen
Hardisty and Wolfe, 1955	66,M	Anemic to 6.0	.....	.....	2,900–41,000	700,000 – 4,100,000	Occasional eosinophilia	No abnormality	26 mo.	Postsplenectomy; priapism
	73,F	.....	.....	.....	Normal	1,450,000 – 2,580,000	Normal	Hypercellular, marked megakaryocytic increase	39 mo.	Died of massive pulmonary embolus, necropsy: no leukemia
Spaët et al., 1956..	71,F	Anemia to slight polycythemia	.....	.....	7,000–45,000	Up to 10,000,000	Normal	Hypercellular, megakaryocytes increased	6	Author's diagnosis: polycythemia vera
Lebon et al., 1957.	34,F	2.7–4.0	.....	.....	1,700– 9,800	2,000,000 – 4,000,000	Marked left shift, erythroblast, megakaryocytes	Basophilia, increased megakaryocytes, large platelet masses	?	Splenic smear, liver biopsy: megakaryocytes and platelets
Lemaire et al., 1957	31,F	2.3–1.12	.....	.....	7,200–16,000	850,000 – 1,100,000	Normal differential	Megakaryocytes increased, erythroid hyperplasia	3	Incompletely studied
Thieffry et al., 1957	12,M	5.11	15.6	40–44	16,400	Normal–4,000,000	Normal	Megakaryocytes increased, platelet masses	30 mo.	Treated with P^{32}
Goudemand and Hutin, 1957	55,M	1.5–5.3	.....	.....	10,400–25,000	420,000 – 3,760,000	Normal	Normal	?	....
La Porte et al., 1957	54,M	4.0–5.1	16.3	52	8,000–16,000	1,000,000 – 2,700,000	Eosinophilia 25%	Hypercellular megakaryocytes increased, platelet masses	?	Postsplenectomy, spleen weighed 1750 gm.
Olivarius, 1957...	58,M	3.5–3.7	74–66	.....	12,000–17,000	1,332,000 – 2,360,000	Normal	Increased megakaryocytes, exceedingly abundant platelets	?	Thrombosis of carotid artery, patient later developed marked anemia with 49% immature white blood cells in periphery, thrombocytopenia; splenic aspiration: stem cells; probably leukemic transformation
Arlotti, 1957.....	66	4.0	62	.....	12,000	700,000 – 900,000	Normal	Not done	3	Incompletely studied
Bousser, 1957.....	53,F	6.12	.....	.....	17,000	2,600,000	Slight left shift, slight eosinophilia	Increased megakaryocytes	31 mo.	Treated with P^{32}
Baikie, 1958.....	51,F	5.5	80	.....	33,000	2,200,000	Granulocytosis	Hypercellular, increased megakaryocytes	3 mo.	Treated with HN_2
Levinson, et al., 1958	19,F	6.38	15.7	49	10,000–45,000	3,000,000 plus platelet masses	Occasional myelocytes, eosinophilia, basophilia, normoblasts	Normal to increased megakarocytes	5 mo.	Necropsy: no evidence of leukemia, pulmonary alveolar proteinosis
Shaw and Oliver, 1958	32,M	Normal	.....	.....	14,000	700,000 – 1,800,000	Not described	Not reported	?	Hemiplegia, epilepsy, peripheral vascular insufficiency
	35,F	Mild anemia	.....	.....	13,000	1,150,000 – 3,830,000	Occasional myelocytes	Not reported	?	Insufficient information
	43,F	Not given	.....	.....	Normal	1,360,000 – 1,560,000	Not given	Not reported	?	Insufficient information
Bindswanger et al., 1955	35,F	3.3–2.85	50–58	.....	18,600–50,000	2,000,000 – 6,000,000	Eosinophilia, basophilia	Not reported	3	Postsplenectomy
Goudsmit, 1956...	76,M	Anemia to above 7.0	8 125	.....	14,500–45,000	1,806,000	Normal differential	Very cellular, remarkable increase in megakaryocytes	2.5	Polycythemia vera

a nineteen year old girl who showed no evidence of leukemia at necropsy, but in whom examination of the lungs demonstrated a picture similar to "pulmonary alveolar proteinosis" as reported by Rosen et al. [*58*]. Other cases that we have been able to review are listed in Table II. The total number, no doubt, exceeds this, since not all the original articles were available to us. Some of these cases were insufficiently studied or the information given was not adequate [*18,24,28,34* (second and third case), *40* (first and second case)]. A number of other cases cannot be regarded as authentic since splenic atrophy [*2,23* (first case), *21*], surgical absence of the spleen (*15,23,29,66*, (second case), *22,38* (third case), *14* (third case)], polycythemia [*5,16,57*] or chronic granulocytic leukemia [*4*] was present. Of these forty-eight cases, only twenty-one [*6* (first case), *7,9,10,12,13,17,49* (three cases), *19,20,22* (two cases), *24,25,27,30,31,32,36*] are examples of primary hemorrhagic thrombocythemia, according to the criteria suggested herein.

Analysis of all the case reports provides a description of an entity with rather distinct clinical features. The disease is most commonly seen in late middle age or old age, although it has occurred in a twelve year old patient. The female sex predominates slightly, but this is probably not a statistical dependable observation. The history reveals manifestations of thrombohemorrhagic phenomena in one form or another; however, these may be slight or even absent [*10,22,24*]. Bleeding characteristically occurs in the skin, nostrils, gums or gastrointestinal tract. Genitourinary bleeding (hematuria or vaginal bleeding) is frequent. Excessive menses have not been reported, although most of the patients were not in the childbearing age group. Prolonged bleeding following surgery, particularly dental extractions, is apt to occur.

Thromboembolic manifestations are less frequent than bleeding. Thrombosis of the carotid artery [*36*], femoral artery [*14*], peripheral arteries [*7*], deep [*9*] and superficial veins, probably cavernous bodies of the penis leading to priapism (our second case), pulmonary infarctions [*10,23*] and hemorrhagic infarction of the adrenal glands [*22*] have all been observed. Steatorrhea [*6*] and recurrent pancreatitis [*7*], described in two patients, could have been the result of repeated thromboses. Besides signs of various bleeding tendencies, the most common physical findings are splenomegaly and hepatomegaly. Lymphadenopathy is unusual.

Anemia is more common but there may be slight erythrocytosis. Leukocytes are moderately increased, with slight eosinophilia and basophilia, and a few immature leukocytes may appear in the differential count. The hallmark of the syndrome, however, is the marked and persistent thrombocythemia. The platelets often show marked morphologic changes [*10,45*]. Occasionally fragments of megakaryocytes are seen in the peripheral blood. The bone marrow almost always reveals a picture of panmyelosis in which megakaryocytes predominate. Large masses of platelets are seen. Mild eosinophilia and basophilia in the bone marrow is common.

The anemia probably results from blood loss. The possibility of an hemolytic component has not been adequately studied. When gastrointestinal or genitourinary bleeding occurs a definite source usually cannot be found, although peptic ulcers have been demonstrated in a few cases [*10,49*]. It would appear that blood oozes from the mucosal surfaces. The tourniquet test usually is not helpful in these cases.

The bleeding tendency, which these patients so frequently exhibit, has not been adequately explained. The platelets in some cases were found to be qualitatively deficient as determined by the thromboplastin generation test [*23,40*]. Bigelow et al. [*59*] found that the content of serotonin of the platelets is below normal in some cases. Spaet et al. [*26*] questioned the significance of the thromboplastin generation test and described anticoagulant activity in normal platelets when concentrated to thrombocythemic levels. These authors suggested that the fundamental defect in hemorrhagic thrombocythemia is a coagulation defect resulting from the anticoagulant effect of excess platelets. The platelets in thrombocythemia are functionally normal, but excessive numbers of platelets impair the process of coagulation both by interfering with thromboplastin formation [*33*] and by inhibiting the action of thromboplastin already formed [*26*]. The low fibrinogen levels found in some patients [*20*] are probably not a sufficient cause of bleeding. In extensive studies Soulier et al. [*60*] showed that marked variations occur from case to case, as well as in the same patient. Increased thromboplastin generation by platelets, increased clotting tendency, increased fragmentation of platelets and decreased proaccelerin activity have been demonstrated.

In one of our patients [*61*] there was a correlation between hemorrhagic tendency and spontaneous reduction in the number of platelets. Simultaneously there occurred a pronounced reduction in prothrombin, accelerator globulin and fibrinogen. This suggested the possibility of intravascular coagulation, with or without thrombus formation, followed by a deficiency of one or more coagulation factors. Hemorrhagic symptoms may be expected to ensue should these factors be reduced to critically low levels.

Not all patients who bled exhibited the same coagulation defect and in some patients no defect could be found by available methods. It is apparent that more studies and the application of other technics are required to explain the hemorrhagic tendency in all cases of thrombocythemia.

For the treatment of essential thrombocythemia, urethane [*13*], x-ray [*49*], nitrogen mustard [*39*] and, more recently, radioactive phosphorus [*25,30,32,39,41,49*] have been used. The latter seems to be the treatment of choice and is administered as in polycythemia vera.

The data are insufficient with respect to evaluation of prognosis. Although patients have been reported living six years after the original diagnosis, death frequently ensues in less than four years. Two cases definitely ([*24*], V. L.) and one case possibly [*36*] terminated with transformation to myelogenous leukemia. With the introduction of P^{32} a longer survival time may be expected. In such an event it seems probable that acute leukemic transformation may be expected to occur more commonly, as in polycythemia rubra vera.

COMMENTS

An increase in the number of platelets in the peripheral blood is generally classified as either primary (thrombocythemia) or secondary (thrombocytosis). The latter is quite common [*43*]. Acute tissue necrosis following trauma or inflammation, particularly following fractures, is often followed by a temporary increase in platelets. Thrombocytosis also commonly accompanies acute blood loss. Splenectomy is one of the well recognized causes of thrombocytosis. Although usually temporary, the thrombocytosis may last several years [*3,42*] and produces a picture indistinguishable from the idiopathic form.

Chronic granulocytic leukemia is not infrequently associated with high levels of platelets [*51*], sometimes over 10 million per cu. mm. (personal observations). Thrombocytosis is common also in polycythemia vera [*44,45*] and agnogenic myeloid metaplasia [*53*]; and occurs rarely in disseminated carcinoma [*8*], tuberculosis [*50*], Boeck's sarcoid, Cushing's syndrome [*52*] (personal observation) and Hodgkin's disease [*63*].

Whether idiopathic thrombocythemia may occur independently, as an entity separate particularly from polycythemia vera or leukemia, is debated [*24*]. However, after eliminating all the "secondary" cases, a group remains which cannot be classified otherwise.

On the basis of a survey of the literature, and our own observations, we propose the following criteria for the diagnosis of primary hemorrhagic thrombocythemia: (1) a history of thrombohemorrhagic phenomena; (2) the presence of a spleen of at least normal size; (3) a peripheral blood picture with (a) erythrocytes not in excess of 6,000,000 per cu. mm., hemoglobin not exceeding 18 gm. per cent, hematocrit not above 54 per cent, (b) leukocytes not in excess of 50,000 per cu. mm., (c) platelets persistently above 800,000 per cu. mm. (indirect method); (4) a bone marrow film showing hyperplasia of all elements (panmyelosis) with predominance of megakaryocytes and with platelet masses; in addition, there may be eosinophilia and basophilia in the peripheral blood and/or bone marrow; and (5) absence of leukemic infiltration as determined by examination of biopsy or necropsy material.

Although thrombohemorrhagic phenomena are usually present, they may rarely be absent. All patients with asplenia, splenic atrophy or thrombosis of the splenic vessels prior to establishment of diagnosis have been excluded. The presence of splenomegaly is helpful in diagnosis and is often observed.

An arbitrary limit of 50,000 leukocytes per cu. mm. is chosen because in chronic granulocytic leukemia the white cell count is only occasionally below this level [*62*] and in primary thrombocythemia only rarely above this level [*41*]. Determination of the alkaline phosphatase activity of the polymorphonuclear cells may be expected to be helpful, although to our knowledge no such determinations have been made in cases of primary thrombocythemia.

An arbitrary limit of 6 million erythrocytes per cu. mm. is chosen as a differential point from

polycythemia vera [*44*], since erythrocytes usually exceed 7 million per cu. mm. in the latter disease.

It should be stressed, however, that primary thrombocythemia is a form of panmyelosis in which elevation of platelets predominates. Only rarely is thrombocythemia the sole abnormality, without leukocytosis and erythrocytosis [*22*]. In this respect it is similar to polycythemia vera and chronic granulocytic leukemia. In the latter disease erythrocytosis is not uncommon, particularly in the early phases. In polycythemia vera, which is another form of panmyelosis, thrombocytosis and leukocytosis are common. The basic similarities of these entities have already been pointed out by Dameshek [*46*] and others [*48*] in discussions of the myeloproliferative syndromes. This concept may also explain the transformation of primary thrombocythemia to acute myeloblastic leukemia, which has been cited as evidence against the entity under discussion [*24*]. This objection cannot be accepted as valid, since more than 20 per cent of cases of polycythemia vera terminate as myelogenous leukemia [*44,54*].

Much of the confusion arises when thrombocytosis is associated with slight erythrocytosis. Admittedly the diagnosis then becomes a matter of preference; we believe that by the criteria herein outlined most cases can be diagnosed correctly.

If anemia is present, there would be some hesitancy to label these cases as polycythemia vera with bleeding, unless at least a history of polycythemia vera had been previously confirmed [*44*]. The concept of primary thrombocythemia then becomes useful for the proper management of the patient.

SUMMARY

1. Six new cases of primary hemorrhagic thrombocythemia are reported. All six patients exhibited thrombohemorrhagic phenomena in one form or another. Purpura, epistaxis, and bleeding of the gums and gastrointestinal tract were common. All had hepatosplenomegaly. In the patient with gastrointestinal bleeding, sources could not be found. All had persistent elevation of thrombocyte count above 800,000/cu. mm. (indirect method). Acute myeloblastic leukemia finally developed in one patient.

2. The literature is critically surveyed. On the basis of this survey and from personal experience new diagnostic criteria are outlined. These include: (a) a history of thrombohemorrhagic phenomena; (b) the presence of the spleen, which is usually enlarged; (c) erythrocyte level of not more than 6 million/cu. mm., hemoglobin not to exceed 18 gm. per cent, hematocrit not above 54 per cent, leukocytes not exceeding 50,000/cu. mm., and a platelet count persistently above 800,000/cu. mm. (indirect method); (d) hyperplasia of all marrow elements with megakaryocytic preponderance and platelet masses plus eosinophilia and/or basophilia; and (e) absence of leukemic tissue infiltration.

3. It is emphasized that primary hemorrhagic thrombocythemia is a myeloproliferative syndrome in which elevation of platelets predominates, thus constituting a separate entity apart from polycythemia vera, chronic granulocytic leukemia and secondary thrombocytoses.

4. The recognition of this entity is clinically important in guiding management. Radioactive phosphorus appears to be the treatment of choice at the present time.

APPENDIX

CASE 1. A twelve year old white girl, V. L. (JSH No. 2886-M), was in good health until January 1953, when she suddenly vomited a large amount of blood. In May 1953 a tonsillectomy had been performed. Although the coagulation time was said to have been normal prior to the operation, there was rather severe bleeding postoperatively. In September 1954 there was marked bleeding following an uncomplicated tooth extraction. In October 1954 the patient began to complain of left costovertebral angle tenderness, fever and dysuria. An abscess of the kidney was suspected and an exploratory laparotomy was performed. However, only splenomegaly was found. Following the operation prolonged bleeding from the operative site occurred, and there was a rather stormy postoperative course. In late January 1955 the patient had a severe nosebleed, and reportedly bled from the site of the operative incision. On February 5, 1955, the patient was admitted to John Sealy Hospital for the first time.

Physical findings on admission revealed the spleen to be palpable 5 cm. below the left costal margin. Laboratory studies on admission yielded the following results: hemoglobin 12.4 gm. per cent, red blood cells 4,400,000 per cu. mm., reticulocytes 1 per cent, platelets 5,552,800 per cu. mm., white blood cells 19,700 per cu. mm., polymorphonuclears 62 per cent, myelocytes B 1 per cent, basophils 11 per cent, eosinophils 5 per cent, lymphocytes 18 per cent, monocytes 4 per cent. The bleeding time was 18

minutes, coagulation time 10.5 minutes. The bone marrow on admission revealed tremendous numbers of megakaryocytes which showed a shift to the left. Results of the tourniquet test were negative.

The peripheral count continued to show marked thrombocythemia, as well as a leukocytosis with basophilia and eosinophilia. Special coagulation studies were performed which were reported as showing intermittent hypoprothrombinemia and fibrinogenopenia. A suggestive correlation was noted between spontaneous falls in the platelet count and low fibrinogen levels. Between March 10 and March 29, 1955, a total of 5.15 mc. of P^{32} was administered intravenously. There was a moderate fall in the platelet count to an average of about 2 million per cu. mm. and no further hemorrhagic episodes ensued.

The patient was discharged and followed on an outpatient basis for the next few months during which time she experienced no further bleeding; however, in August 1955 the platelet count again reached high levels, and she was readmitted for the purpose of administering P^{32}. On physical examination, the spleen was found to be 8 cm. below the left costal margin, and the liver 4 cm. below the right costal margin. Laboratory studies on August 30 showed a hemoglobin of 12.4 gm. per cent, red blood cells 4,210,000 per cu. mm., reticulocytes 2.8 per cent, white blood cells 20,250 per cu. mm., polymorphonuclears 77 per cent, stab forms 1 per cent, basophils 4 per cent, eosinophils 3 per cent, lymphocytes 15 per cent, platelets 5,144,620 per cu. mm. The bleeding time was 7 minutes. Between September 10 and October 1, 1955, a total of 7.5 mc. of radioactive phosphorus was administered. Following this there was a fall in the leukocyte count to normal levels, and the platelets gradually declined to lower, although still elevated, levels. Following discharge on October 5, 1955, she was asymptomatic until April 6, 1956, when she had a profuse nosebleed. She was seen in the outpatient clinic two weeks later, and was admitted to the hospital for further treatment on April 23.

As before, physical examination revealed hepatosplenomegaly. The blood count on admission was as follows: hemoglobin 12.3 gm. per cent, red blood cells 4,930,000 per cu. mm., platelets 4,491,230 per cu. mm., white blood count 22,500 per cu. mm., polymorphonuclears 75 per cent, stab forms 4 per cent, metamyelocytes 1 per cent, myelocytes B 1 per cent, myelocytes A 2 per cent, basophils 2 per cent, eosinophils 2 per cent, lymphocytes 13 per cent. The bleeding time was 15 minutes. It was decided to give the patient another course of radioactive phosphorus and between May 10 and May 22, 1956, she received a total of 9 mc. of P^{32}. Following this her platelet count fell to approximately 3 million per cu. mm. She was then discharged and remained well until August 1956 when she had "pneumonia" at her home town. Early in October 1956 she began to complain of weakness and aching in the legs. She was readmitted on October 31, 1956.

Findings on physical examination were unchanged, as were the peripheral blood counts. (Fig. 3.) However, examination of the bone marrow revealed predominant myeloblasts and early myelocytes. Megakaryocytes were still numerous. During the second week of November 1956 she received a total of 7 mc. of P^{32}. Another bone marrow aspiration performed in December 1956 showed marked involvement with myeloblasts and myelocytes. She died early in 1957 in her local town. Autopsy was not performed.

Case 2. A sixty year old Latin-American man, B. G. (JSH No. 18053-J), was first admitted to the John Sealy Hospital on February 25, 1954, with multisystem complaints including weakness, exertional dyspnea, swelling of the legs, postprandial fullness of the stomach, epigastric pain, tarry stools and occasional priapism of five months' duration. The pertinent physical findings included marked pallor, hepatomegaly and splenomegaly. There was no significant lymphadenopathy. The initial blood count was as follows: hemoglobin 4.7 gm. per cent, red blood cells 2,930,000 per cu. mm., white blood cells 11,600 per cu. mm. with polymorphonuclears 75 per cent, lymphocytes 10 per cent, monocytes 9 per cent, basophils 5 per cent, eosinophils 1 per cent. The reticulocyte count was 5 per cent. The platelets numbered 1,582,000 per cu. mm. The erythrocytes showed marked hypochromia and microcytosis. The stools were persistently guaiac-positive. Roentgenologic examination of the gastrointestinal tract, sigmoidoscopy, gastroscopy, intravenous pyelogram, liver biopsy, splenic aspiration, muscle biopsy, bleeding, clotting and one-stage prothrombin time were essentially within normal limits. A bone marrow aspiration revealed hyperplasia of all the elements, particularly of the megakaryocytes, and large masses of platelets were seen. Repeated blood counts remained unchanged, the platelet count ranged from 1.5 to 3.9 million per cu. mm. The significance of the latter finding, however, was not appreciated at the time and the patient was discharged with a diagnosis of "iron deficiency anemia of unknown cause." He was given oral iron therapy, with good initial response.

The patient was not seen again until January 30, 1956, at which time he returned to the outpatient clinic with the same complaints. The physical findings were unchanged. A peripheral blood count was reported as follows: hemoglobin 5.9 gm. per cent, red blood cells 4,060,000 per cu. mm., white blood cells 12,350 per cu. mm., with polymorphonuclears 81 per cent, stab forms 1 per cent, lymphocytes 2 per cent, monocytes 4 per cent, basophils 9 per cent, eosinophils 3 per cent, reticulocytes 3.4 per cent, and platelets 1,965,000 per cu. mm. The stools again were persistently guaiac-positive. Roentgenologic and endo-

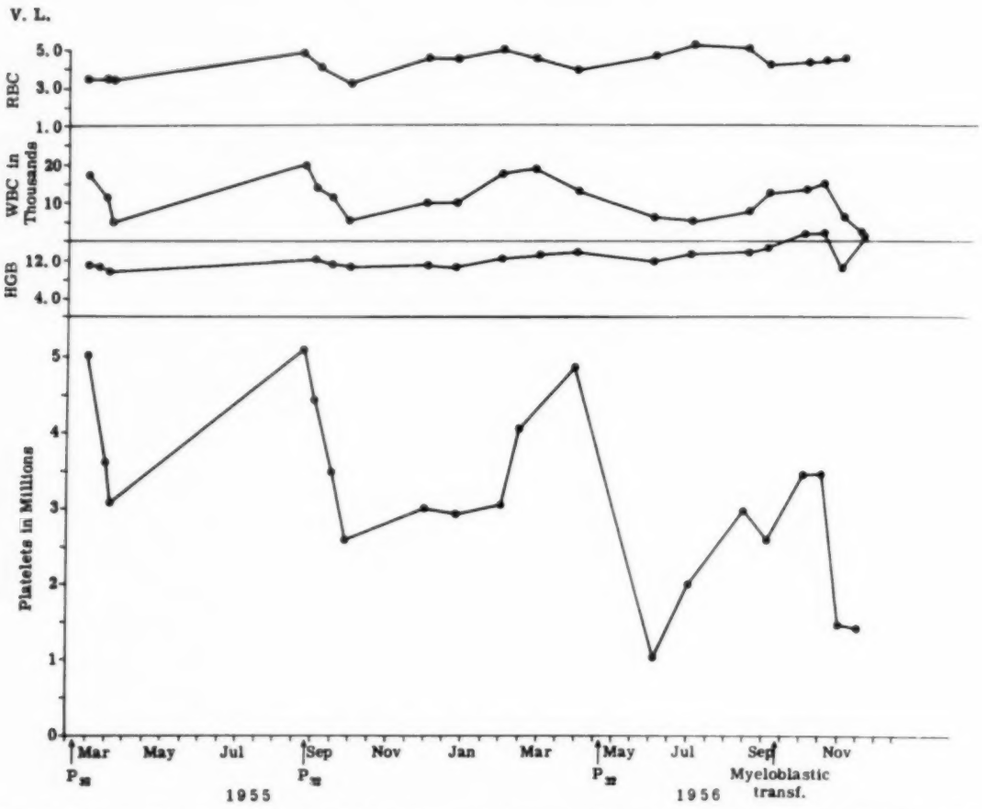


FIG. 3. Case 1. Course of peripheral blood counts. Arrows indicate P^{32} treatment. (Red blood cells in millions, hemoglobin in gm. per cent, white blood cells in thousands, platelets in millions.)

scopic examinations this time also were non-revealing. The serum iron was less than 25 μg. per cent. The hemolytic index was 31 (normal up to 22). A bone marrow aspiration revealed hypercellularity with strikingly increased megakaryocytes and large masses of platelets.

Approximately six weeks after his admission arterial occlusion of the left foot developed. Paravertebral sympathetic blocks were effective; therefore, an emergency left lumbar sympathectomy was performed through an abdominal approach on March 27, 1956. During the operation 1,000 cc. of whole blood was transfused. The operative and postoperative course was uneventful. Beginning on April 4, 9 mc. of P^{32} was administered intravenously over a period of three weeks. The hematologic response is depicted in Figure 4. On June 18, 1956, a transurethral resection of the prostate and on July 2 a transmetatarsal amputation of the left foot were performed without difficulty.

CASE 3. A fifty-three year old white woman, L. E. (JSH No. 14270-J), was first seen in the outpatient clinic on September 23, 1953. Her chief complaint was severe burning of the skin for one and a half years. In addition, she had had multiple non-specific gastrointestinal complaints and a sleep disturbance for several years. Physical examination was essentially within normal limits except for palmar erythema and a maculopapular eruption localized on the upper part of the arm. A peripheral blood count revealed the following: hemoglobin 16.3 gm. per cent, white blood cells 13,800 per cu. mm., with polymorphonuclears 85 per cent, lymphocytes 13 per cent, monocytes 1 per cent, eosinophils 1 per cent. On January 18, 1956, she was seen again in the outpatient clinic, with the same complaints. Rectal bleeding and several episodes of spontaneous epistaxes had occurred in 1955. On physical examination there was marked pallor. The liver was 2 fingerbreadths below the right costal margin. The spleen was felt 1 cm. below the left costal margin. The peripheral blood count was as follows: hemoglobin 6.3 gm. per cent, red blood cells 2,950,000 per cu. mm., white blood cells 26,750 per cu. mm. with polymorphonuclears 76 per cent, stab forms 3 per cent, metamyelocytes 3 per cent, lymphocytes 9 per cent, monocytes 3 per cent, basophils 4 per cent, eosinophils 4 per cent, reticulocytes 3.6 per cent, platelets 3,440,700 per cu. mm. The mean corpuscular volume was 93 cu.μ., mean corpuscular hemoglobin 21, mean corpuscular hemoglobin concentration 23 per cent. Bleeding and clotting times were normal. The

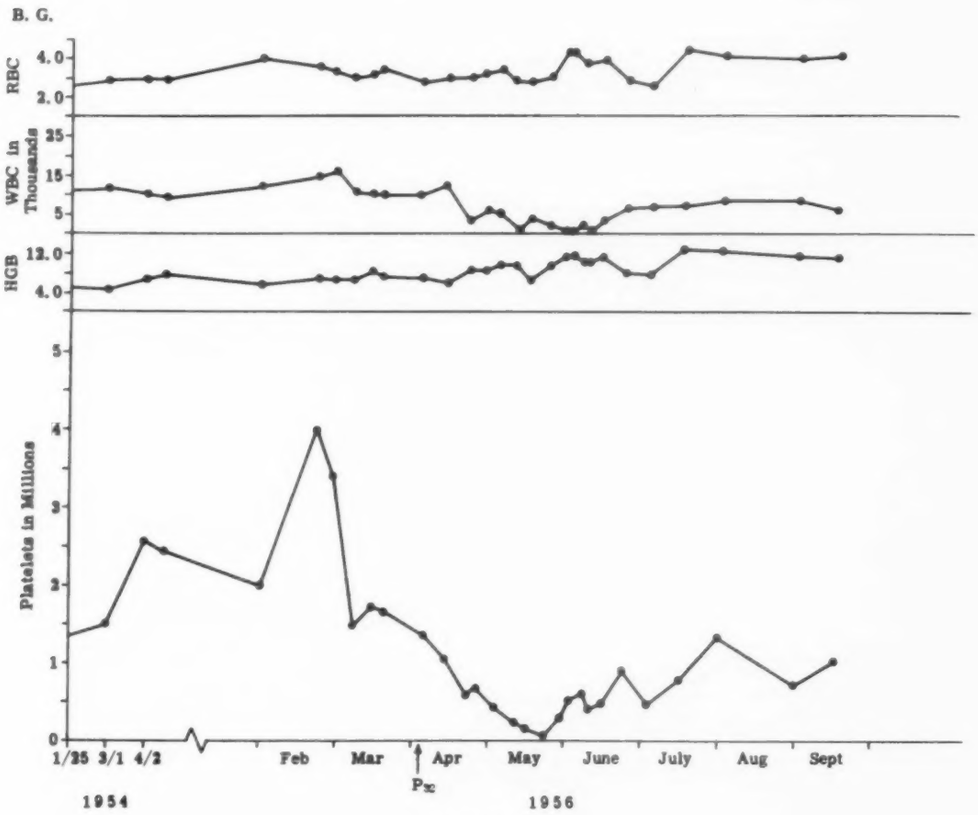


FIG. 4. Case 2.

serum iron was less than 25 μg. per cent. A bone marrow aspiration revealed marked hypercellularity of all the elements, with eosinophilia. The megakaryocytes were strikingly increased and large platelet masses were seen in all fields. Several stool specimens were guaiac-positive; however, no definite source of bleeding could be found on x-ray, sigmoidoscopic or gastroscopic studies.

Between March 26 and April 10, 1956, she was given 9.5 mc. of P^{32} intravenously in divided doses. Ferrous iron also was prescribed. Within four weeks a hematological remission occurred. (Fig. 5.) There was marked clinical improvement, with disappearance of her complaints. The spleen and liver could no longer be palpated. Bone marrow examination on April 23, 1956, revealed a definite decrease in the number of megakaryocytes. Her remission continued until September 1956 at which time her previous complaints reappeared, with an increase in platelets, leukocytes, and to some extent, erythrocytes and hemoglobin.

On October 10, 1956, she was readmitted to the hospital. Physical examination was within normal limits. Over a two-week period 9 mc. of P^{32} was administered. She was in clinical and hematologic remission when she was seen on April 30, 1957.

CASE 4. This fifty-two year old Latin-American man, F. A. (JSH No. 34380-M), was in good health until 1954, at which time he vomited small amounts of blood; this was associated with epigastric pain. He was treated with an ulcer regimen by his local physician, which resulted in relief of his symptoms. In 1955 he began to have uncinate fits, and later Jacksonian seizures. Antiepileptic therapy was instituted. Following this, gingival bleeding occurred with slight trauma. The past history revealed that he had had syphilis which was treated with arsenicals and penicillin.

He was admitted to the Neurology Service on December 11, 1956, because of the seizures. A pneumoencephalogram, bilateral carotid arteriograms and electroencephalogram were within normal limits. The hemoglobin was 17.5 gm. per cent and hematocrit 54 per cent. The blood pressure was 168/110 mm. Hg. Slight hepatosplenomegaly was noted. A hemogram revealed the following: hemoglobin 16.4 gm. per cent, white blood cells 11,600/cu. mm., polymorphonuclears 60 per cent, stab forms 6 per cent, lymphocytes 13 per cent, monocytes 13 per cent, basophils 2 per cent, eosinophils 4 per cent. The cell volume was 54 per cent. A bone marrow aspiration revealed a hypercellular marrow. There

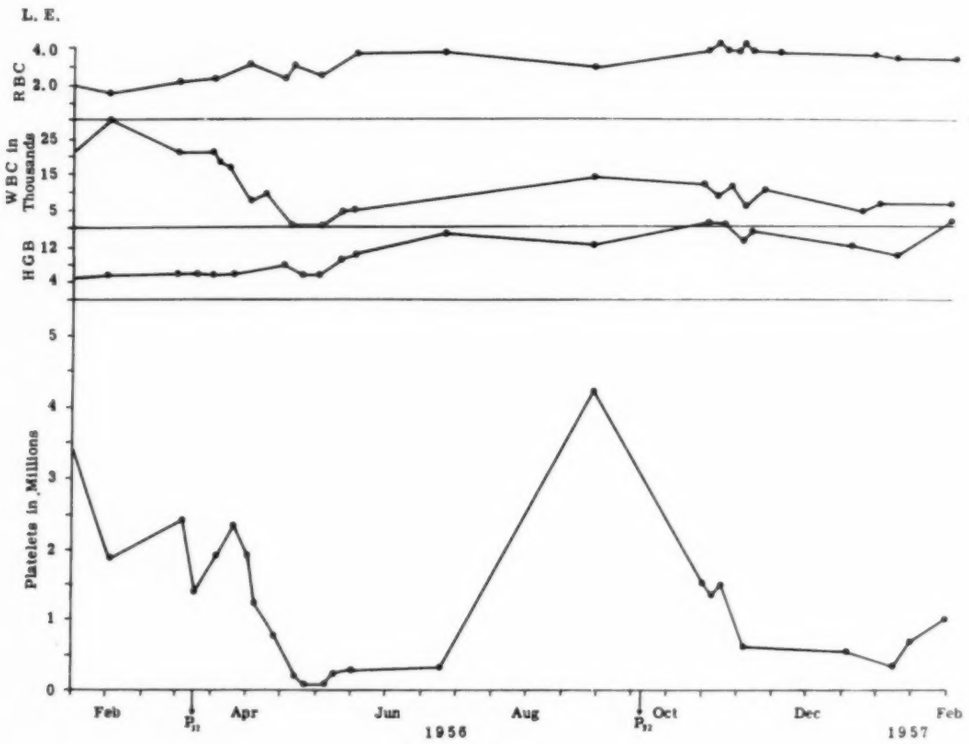


FIG. 5. Case 3.

was profound megakaryocytic hyperplasia. Platelet counts ranged between 1,073,000 and 3,599,000 per cu. mm. A tourniquet test was strongly positive. Detailed coagulation studies gave results within normal limits.

Between January 14 and 24, 1957, he received 6.7 mc. of P^{32} in divided doses. As can be seen in Figure 6, a hematologic remission occurred one month after therapy with P^{32} therapy. The bone marrow at this time was normocellular. The megakaryocytes were decreased, but still numerous. The hematologic remission lasted about ten months. During this period, epileptic seizures were less frequent. Beginning in March 1958 the patient noted that he bruised easily. He complained of vague joint pains and his seizures became more frequent. After four months of observation a second course of P^{32} therapy (total 6.5 mc.) was given. There have been no substantial changes in the clinical and hematologic picture.

CASE 5. This fifty-two year old white man, L. B. (JSH No. 39953-M), was referred to the John Sealy Hospital because of subcutaneous nodules and hypertension. Early in 1952, his abdomen had been injured by a steering wheel and had been sore for a week or two afterwards. Later he became weak and began to sweat excessively. He had some discomfort in the left upper quadrant, was nauseated and vomited several times. In July 1952 he discovered a "lump" in his abdomen and visited a surgeon. On physical examination a huge "mass" was found in the left upper quadrant, extending below the umbilicus. The liver was not felt. Results of routine studies and liver function studies were said to be normal. A presumptive diagnosis of "pseudocyst of the pancreas" was made and he underwent laparotomy. The "mass" proved to be the spleen, which weighed 1,000 gm. The pathologic diagnosis was congestive splenomegaly with slight megakaryocytic hyperplasia. A biopsy specimen of the liver showed normal architecture and a lymph node revealed slight reticulum cell hyperplasia. On the night of the operation the patient went into shock, presumably due to hemoperitoneum, and he was treated with blood transfusions.

One month after the operation he began to have uncinate fits and noted easy bruising. In 1953 he experienced episodes of spontaneous epistaxes sometimes lasting three days. Peripheral blood films were reviewed by a hematologist who reported "granulocytic leukocytosis with marked eosinophilia and rather marked increase in platelets; white blood count was 20,000 per cu. mm. and hemoglobin 13.2 gm. per cent."

In 1954 he was admitted to a hospital following an episode of hematemesis. On physical examination generalized lymphadenopathy and moderate hepatomegaly were found. A blood count was as follows:

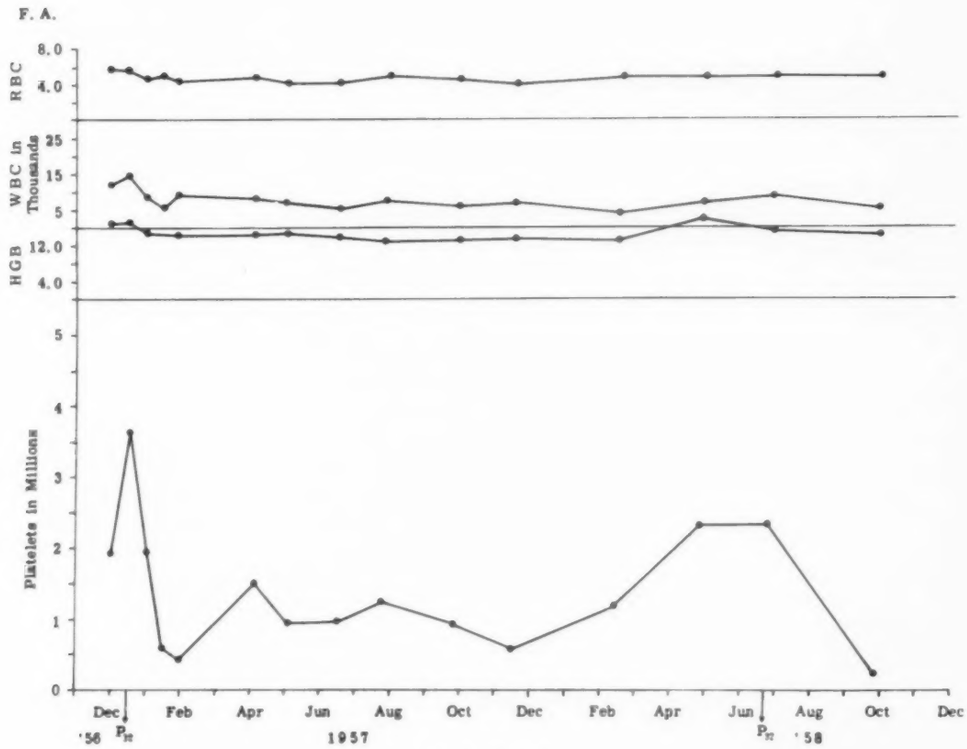


FIG. 6. Case 4.

Hemoglobin 15.8 gm. per cent, white blood cells 24,200 per cu. mm. with polymorphonuclears 60 per cent, stab forms 15 per cent, lymphocytes 21 per cent, eosinophils 14 per cent, platelets 1,250,000 per cu. mm. A lymph node biopsy revealed moderate reticuloendothelial hyperplasia. Despite extensive investigation, a diagnosis could not be established.

In 1956 the patient complained of headaches, vertigo, and pain and burning in the feet. A small area of ulceration developed on one toe. In August 1957 he was admitted to the same hospital following profuse hematuria and marked swelling of the right thigh. Again studies were non-revealing. The same year he experienced spontaneous gingival bleedings. A tooth was extracted, and he bled for two days thereafter. Three weeks prior to his admission to the John Sealy Hospital, he had a spontaneous subconjunctival hemorrhage. His physician also found small subcutaneous nodules.

The pertinent physical findings on admission included a blood pressure of 190/110 mm. Hg, slight axillary lymphadenopathy, a well healed surgical scar in the left upper quadrant, moderate hepatomegaly, absence of left dorsalis pedis pulsation and two small subcutaneous nodules in the left thigh and right pretibial area. The initial hemogram was as follows: hemoglobin 11 gm. per cent, red blood cells 4,000,000 per cu. mm., white blood cells 28,300 per cu. mm. with polymorphonuclears 79 per cent, metamyelocytes 1 per cent, lymphocytes 4 per cent, monocytes 8 per cent, eosinophils 1 per cent, platelets 1,600,000 per cu. mm. A tourniquet test was negative. Detailed coagulation studies were within normal limits. A bone marrow aspiration revealed an increase in myeloid elements with marked eosinophilia. The most striking change was the presence of large amounts of platelet masses and an increase in the number of megakaryocytes.

A routine electrocardiogram showed changes compatible with acute myocardial infarction, in the absence of clinical symptomatology. An electroencephalogram was interpreted as showing generalized disturbance in cerebral cortical activity. Serum total proteins, calcium, phosphorus, alkaline phosphatase, bilirubin, cephalin flocculation, thymol turbidity, non-protein nitrogen, fasting blood sugar, uric acid, bromsulphalein, serological tests, L.E. phenomenon, Sia test were either negative or within normal limits. Phenolsulfonphthalein excretion was moderately decreased. Biopsy of the subcutaneous nodules, which yielded fibro-fatty tissue, was complicated by large hematomas involving the entire left thigh and lower part of the right leg. Blood continued to ooze from the sites of incisions. Bleeding persisted despite tight dressing, intravenous administration of vitamin K_1 oxide, and topical application of com-

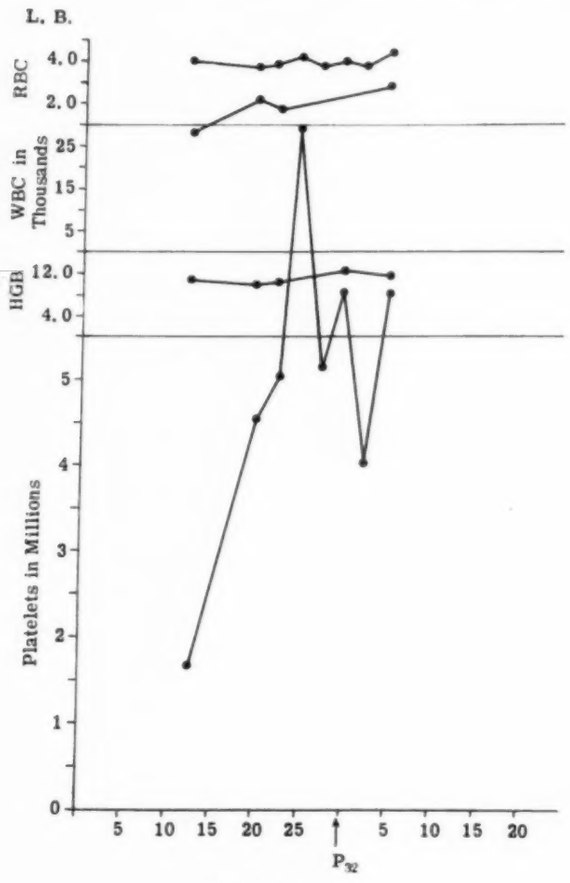


FIG. 7. Case 5.

mercial thrombin, but ceased spontaneously after several days.

Subsequent blood counts (Fig. 7) showed marked leukocytosis (up to 41,000) with marked eosinophilia (41 per cent). The platelet count ranged between 4,519,500 and 13,842,700 per cu. mm. During the period of hemorrhage from the biopsy sites the one-stage prothrombin test was below 10 per cent of normal.

The patient was treated with 6 mc. of P^{32}, given intravenously in a single dose. Three days later he left the hospital against advice. Follow-up studies are not available.

Comment: This case cannot be definitely classified as primary hemorrhagic thrombocythemia, since splenectomy had been performed. It is included, however, because of the remarkable symptomatology which this patient exhibited. Moreover, no explanation could be found for the splenomegaly and it is likely that prior to splenectomy he had a myeloproliferative disease which later became more apparent.

CASE 6. A fifty-eight year old white woman, R. E. (JSH No. 52805-M), was seen in the outpatient clinic because of bleeding gums. About five years before she had consulted her physician because of severe headaches and dizziness. The blood pressure was found to be elevated. Two or three years later she noticed slight bleeding of the gums, for which she received vitamin C and "livershots," and was advised to have all her teeth extracted. She had two teeth extracted, followed by profuse bleeding for about eight hours. Shortly thereafter, she experienced episodes of dizziness with a tendency to fall, weakness of the right side of the body, and dysarthria lasting several minutes. During the past two to three years there were three episodes of hematuria with severe renal colic. Vaginal spotting had occurred one to three times each year since 1955, although menopause had occurred ten years previously. Angina of effort was apparent for five years and intermittent claudication for three years. Recently she had had a sensation of burning in her feet and tingling in the tips of the fingers. One year before she noticed purpura with either minimal or no trauma. Since December 1957 profuse gingival bleeding has increased. Family history revealed hypertension and carcinoma in several members of her family.

The pertinent physical findings included a blood pressure of 200/100 mm. Hg, maculopapular erythematous rash of the face, large areas of ecchymoses over the extremities, beefy, red gingiva and moderate splenomegaly.

The initial blood count was as follows: hemoglobin 16.8 gm. per cent, red blood cells 6,200,000 per cu. mm., white blood cells 20,150 per cu. mm., with polymorphonuclears 77 per cent, lymphocytes 11 per cent, monocytes 6 per cent, basophils 2 per cent, eosinophils 4 per cent, platelets 6,940,000 per cu. mm., reticulocytes 1.6 per cent, hematocrit 54 per cent, mean corpuscular volume 92, mean corpuscular hemoglobin 27.1, mean corpuscular hemoglobin concentration 29.5. A bone marrow aspirate consisted almost entirely of platelets. (Fig. 3.) A repeat aspiration revealed slight myeloid hyperplasia. All fields were filled with large platelet masses. The megakaryocytes were increased. Urinalysis, serological tests, chest roentgenogram, electrocardiogram, fasting blood sugar, blood urea nitrogen, cholesterol, total serum proteins, bilirubin, cephalin flocculation, thymol turbidity, uric acid, protein-bound iodine and coagulation studies were either normal or negative. The blood volume was 4,430 cc., the red cell mass 2,380 cc. The half-life of the red cells was twenty-six days (CR^{51}). Subsequent blood counts were similar. Platelet counts ranged between 2,662,000 and 8,970,000 per cu. mm. (Fig. 8.) Between May 13 and June 5, 1958, a total of 7.5 mc. of P^{32} was administered in fractional doses. On June 17, 1958, the spleen was not palpable. She was completely asymptomatic and remained so until the present.

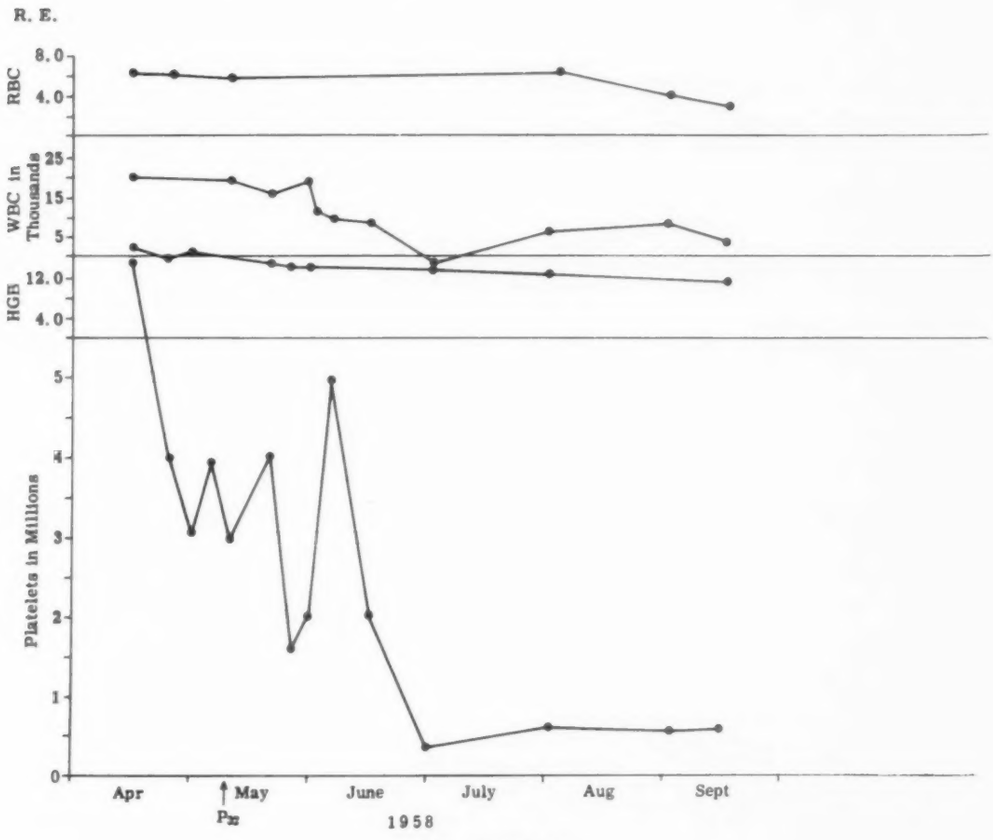


FIG. 8. Case 6.

CASE 7. This forty-nine year old white woman, H. H. (JSH No. 41823-M), was referred to the Neurology Service of John Sealy Hospital because of "brain tumor." As she was mentally unclear, the history was obtained from her husband and daughter. About five weeks prior to admission, the patient complained of cramping epigastric pain. Two or three days later she vomited two cupfuls of dark blood and then passed tarry stools. About this time she complained of severe headaches, chills and fever. One week later she coughed up blood-streaked sputum and passed bloody purulent material through the nose. Shortly thereafter a right-sided hemiparesis gradually developed and she experienced a "Jacksonian" seizure. During the two-month-period prior to admission she had been noted to bruise easily and had a large ecchymosis on one arm. On admission April 12, 1958, the pertinent physical findings included a temperature of 100.2°F., crusted blood in the nose, a large area of ecchymosis on the right arm, moderate hepatomegaly, moderate to marked splenomegaly, and right-sided hemiparesis. The blood count was as follows: hemoglobin 10.3 gm. per cent, white blood cells 14,200 per cu. mm. with polymorphonuclears 87 per cent, stab forms 2 per cent, lymphocytes 7 per cent, monocytes 3 per cent, eosinophils 1 per cent. Roentgenograms of the skull and gastrointestinal tract revealed no abnormalities. Examination of the cerebrospinal fluid was within normal limits. An electrocardiogram was normal. Three days later her blood count was as follows: hemoglobin 5 gm. per cent, red blood cells 2,190,000 per cu. mm. with polymorphonuclears 79 per cent, lymphocytes 6 per cent, monocytes 2 per cent, basophils 2 per cent, eosinophils 1 per cent, reticulocytes 2.6 per cent, platelets 2,417,760 per cu. mm., cell volume 20 per cent, mean corpuscular volume 91.4, mean corpuscular hemoglobin 22.8, mean corpuscular hemoglobin concentration 25 per cent. A bone marrow specimen revealed intense hypercellularity of all the elements. The eosinophils were increased. The erythroid series was megaloblastic. The megakaryocytes were greatly increased. Platelets were present in large masses. The patient was given a transfusion of 1,000 cc. whole blood and transferred to the Medical Service. Physical examination at this time revealed a temperature of 102.2°F. and signs of pulmonary consolidation. The liver was enlarged and the spleen was palpable at the iliac crest. The neurologic findings were unchanged. Antibiotic administration was followed by abatement of fever. Thrombocythemia, however, persisted. (Fig. 9.) Due to the acuteness of her condition, 10 mg. of nitrogen mustard was administered intravenously on May 4, 1958. On May 5, thrombophelebitis of the

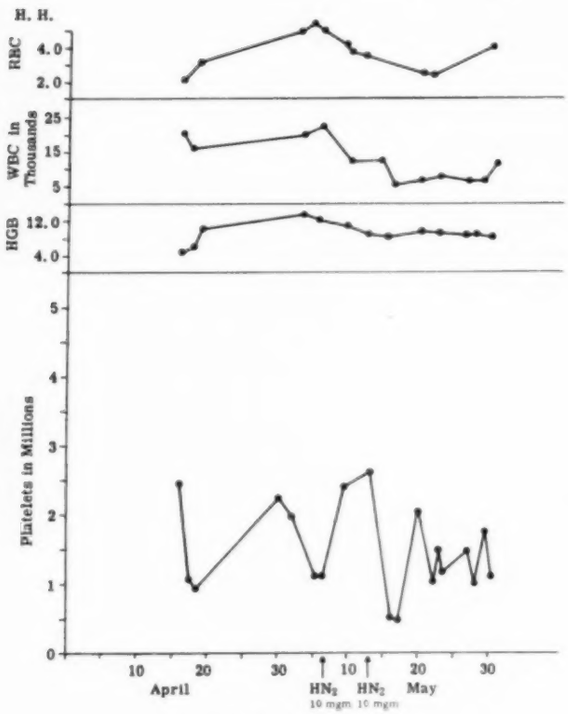


FIG. 9. Case 7.

lower right leg developed which was treated conservatively. Five days later 10 mg. of nitrogen mustard was again administered.

Urinalysis showed slight proteinuria, with low specific gravity. The urinary output was very high (8,000 to 11,000 cc. per day). An intravenous pyelogram was normal. Liver function studies were within normal limits. On May 22 she was ambulated. One week later she fell while walking and a right hemiparesis developed which lasted a few hours. Later she lapsed into coma; she died on May 31, 1958. Postmortem examination was performed two hours after death. It revealed massive cerebral hemorrhage. The hypothalamus was intact. There was no evidence of leukemia. The final pathologic diagnoses were cerebral hemorrhage and myeloproliferative syndrome.

REFERENCES

1. DI GUGLIELMO, G. Eritroleucemia e piastrinemia. *Folia Med.*, 1: 36, 1920.
2. EPSTEIN, E. and GOEDEL, A. Hämorrhagische Thrombocythämie bei vasculärer Schrumpfmilz. *Virchows Arch. Path. Anat.*, 292: 233, 1934.
3. BRUGSCH, H. Persistierende Thrombocythämie und Leukämie nach Milzentfernung. *Folia haemat.*, 49: 454, 1933.
4. DRAKE, C. E. Leukemia with thrombocytosis. *J. A. M. A.*, 106: 1005, 1936.
5. UOTILA, U. On hemorrhagic thrombocythemia. *Acta med. scandinav.*, 95: 136, 1938.
6. ROWLANDS, R. A. and VAIZEY, J. M. Primary thrombocythaemia. *Lancet*, 2: 1217, 1938.
7. NYGAARD, K. K. and BROWN, G. E. Essential thrombophilia. *Arch. Int. Med.*, 59: 82, 1937.
8. AKAZAKI, K. and HAMAGUCHI, I. Ein Fall von Thrombocythämie. *Beitr. path. Anat.*, 103: 95, 1939.
9. REID, J. Haemorrhagic thrombocythaemia. *Lancet*, 2: 584, 1940.
10. OWREN, P. Thrombocythemie essentielle. *Gastroenterologia*, 68: 148, 1943.
11. LEBEL, A. H. Case of hemorrhagic thrombocythemia. *Nord. med.*, 33: 303, 1947.
12. HOLST, J. E. Hemorrhagical thrombocythemia. *Acta med. scandinav.*, 130: 507, 1948.
13. HEMMELER, G. Leucémie mégakaryocytaire avec thrombocythémie. *Schweiz. med. Wchnschr.*, 78: 976, 1948.
14. MOOLTEN, S. E., VROMAN, L., VROMAN, G. M. S. and GOODMAN, B. Role of blood platelets in thromboembolism. *Arch. Int. Med.*, 84: 667, 1949.
15. SPÅNBERG, J. and ZETTERGREEN, L. A case of thrombocythemia essentialis. *Acta med. scandinav.*, 135: 176, 1949.
16. MORTENSEN, O. Thrombocythemia hemorrhagica. *Acta med. scandinav.*, 129: 547, 1948.
17. JASINSKI, B. Zur Pathogenese der Thrombocythämie. *Acta haemat.*, 2: 145, 1949.
18. JACKSON, D. P., HARTMANN, R. G. and CONLEY, L. Clot retraction as a measure of platelet function. II. Clinical disorders associated with qualitative platelet defect. *Bull. Johns Hopkins Hosp.*, 93: 370, 1953.
19. MONTO, R. W. Essential thrombophilia. *Arch. Int. Med.*, 90: 54, 1952.
20. SÖDERSTROM, S. On hemorrhagic thrombocytosis. *Nord. med.*, 29: 47, 1946.
21. FORSELL, J. Polycythemia, anemia and calcification of the spleen. *Nord. med.*, 22: 1123, 1944; 33: 313, 1947.
22. FANGER, H., CELLA, L. J., JR. and LITCHMAN, H. Thrombocythemia. *New England J. Med.*, 250: 456, 1954.
23. HARDISTY, R. M. and WOLFF, H. H. Haemorrhagic thrombocythaemia: a clinical and laboratory study. *Brit. J. haemat.*, 1: 390, 1955.
24. MCCABE, W. R., BIRD, R. M. and MCLAUGHLIN, R. A. Is primary hemorrhagic thrombocythemia a clinical myth? *Ann. Int. Med.*, 43: 182, 1955.
25. WOODROW, J. C. and COPE, S. Haemorrhagic thrombocythaemia treated with radioactive phosphorus. *Brit. M. J.*, 2: 1068, 1955.
26. SPAET, T. H., BAUER, D. and MELAMED, S. Hemorrhagic thrombocythemia, a blood coagulation disorder. *Arch. Int. Med.*, 98: 377, 1956.
27. LEBON, J. Une observation de thrombocythémie (leucémie à mégakaryocytes). *Sang*, 28: 239, 1957.
28. LEMAIRE, A., DEBRAY, J. and COMBRISSON-LEBOLLOCH. Étude clinique, cytologique et biologique d'un cas thrombocythémie chronique avec anémie. *Sang*, 28: 273, 1957.
29. LAPORTE, A. J., MALLARME, J., JACQUOT, A. and COHEN-SOLAL, J. Thrombocythémie hémorragipare post-splénectomé. *Sang*, 28: 226, 1957.
30. THIEFFREY, S., BUHOT, S. and AIGARDI, J. Une observation de thrombocythémie essentielle. *Sang*, 28: 264, 1957.

31. Goudemand and Hutin. Thrombocythémie essentielle. *Sang*, 28: 267, 1957.
32. Bousser, J. Les thrombocythémies. *Presse méd.*, 65: 347, 1959.
33. Bounameaux, Y. Réchèrches sur les méchanisme de la formation de la thromboplastine sanguine. *Acta haemat.*, 17: 65, 1957.
34. Arlotti, O. and Ballerini, G. La patogenesi delle sindromi emorragiche e thrombotiche nelle thrombocitemie. *Haematologica*, 42: 1279, 1957.
35. D'antuono, G. Le thrombocitemie. *Haematologica*, 42: 513, 1957.
36. Olivarus, B. S. Cerebral manifestation in thrombocythemia. *Acta psychiat. et neurol. scandinav.*, 32: 77, 1957.
37. Levinson, B., Jones, R. S., Wintrobe, M. M. and Cartwright, G. E. Thrombocythemia and pulmonary intra-alveolar coagulum in a young woman. *Blood*, 13: 959, 1958.
38. Kupfer, H. G., Ebbels, B. J., Miller, J. N., Thoma, G. W. and Russi, S. Essential thrombocythemia. *Ann. Int. Med.*, 48: 685, 1958.
39. Baikie, A. G., Cunningham, A. T. and McAlpine, S. G. Thrombocythaemia and thrombocytosis in the myeloproliferative syndromes. *Scottish M. J.*, 3: 26, 1958.
40. Shaw, S. and Oliver, R. A. M. Primary haemorrhagic thrombocythaemia. *Proc. Roy. Soc. Med.*, 51: 768, 1958.
41. Fountain, J. R. Haemorrhagic thrombocythemia. *Brit. M. J.*, 2: 216, 1958.
42. Rosenthal, N. Clinical and hematologic studies on Banti's disease. *J. A. M. A.*, 84: 1887, 1925.
43. Tocantins, L. M. The mammalian blood platelets in health and disease. *Medicine*, 17: 155, 1938.
44. Lawrence, J. Polycythemia. Physiology, Diagnosis and Treatment. New York, 1955. Grune & Stratton.
45. Stefanini, M. and Dameshek, W. The Hemorrhagic Disorders. New York, 1955. Grune & Stratton.
46. Dameshek, W. Some speculation on the myeloproliferative syndromes. *Blood*, 6: 372, 1951.
47. Bindswanger, D., Schaub, F. and Scheitlin, W. Thrombocythaemia haemorrhagica. *Acta haemat.*, 14: 382, 1955.
48. Hutt, M. S. R., Pinniger, J. L. and Wetherley-Mein, G. The myeloproliferative disorders with special reference to myelofibrosis. *Blood*, 8: 295, 1953.
49. Stenstrom, N. M. Three cases of megakaryocytic leukemia. *Acta med. scandinav.* (supp.), 246: 234, 1950.
50. Bougton, G. A. and Weinstein, L. Tuberculosis with thrombocythemia: report of a fatal case with necropsy findings. *Boston M. Quart.*, 7: 45, 1956.
51. Minot, G. R. and Buckman, T. E. Blood platelets in the leukemias. *Am. J. M. Sc.*, 169: 477, 1925.
52. Leitner, S. J. Über Thrombocythämien mit megakaryozyte vermehrung im Knochenmark. *Acta med. scandinav.*, 119: 331, 1945.
53. Linman, J. W. and Bethell, F. H. Agnogenic myeloid metaplasia. *Am. J. Med.*, 22: 107, 1957.
54. Quick, A. Hemorrhagic Diseases. Philadelphia, 1957. Lea & Febiger.
55. Goudemand. Les thrombocythémies essentielle. *Semaine hôp. Paris*, 33: 21, 1957.
56. MacPherson, A. I. S., Ingram, G. I. C. and Maclean, N. Thrombocythemia associated with cavernous transformation of the portal vein and hepatomegaly. *J. Roy. Coll. Surgeons, Edinburgh*, 2: 191, 1957.
57. Goudsmit, R. Essential thrombocythemia, chronic myeloid leukemia or polycythemia vera. *Nederl. tijdischr. geneesk.*, 100: 1236, 1956.
58. Rosen, S. H., Castleman, B. and Leibow, A. Pulmonary alveolar proteinosis. *New England J. Med.*, 238: 1123, 1958.
59. Bigelow, F. S. Serotonin activity in blood. Measurement in normal subjects and in patients with thrombocythemia hemorrhagica and other hemorrhagic states. *J. Lab. & Clin. Med.*, 43: 759, 1954.
60. Soulier, J. P., Alagille, D. and Larrieu, M. J. Physiopathologie des hemorrhagies au cours de hyperpléquettoses. *Sang*, 28: 277, 1957.
61. Levin, W. C., Celander, D. R. and Guest, M. The mechanism of hemorrhagic manifestations associated with thrombocythemia. *J. Lab. & Clin. Med.*, 46: 930, 1955.
62. Dameshek, W. and Gunz, F. Leukemia. New York, 1958. Grune & Stratton.
63. Bunting, G. H. The blood picture in Hodgkin's disease. *Bull. Johns Hopkins Hosp.*, 25: 173, 1914.

A Simplified Closed Circuit Helium Dilution Method for the Determination of the Residual Volume of the Lungs*

GEORGE R. MENEELY, M.D., CON O. T. BALL, ROSS C. KORY, M.D.,†
JAMES J. CALLAWAY, M.D., JOSEPH M. MERRILL, M.D.
R. E. MABE, M.D.,‡ DAN C. ROEHM, M.D. *and*
NOLAN L. KALTREIDER, M.D.
Nashville, Tennessee

IN 1941 [*1*] the use of helium was introduced for the purpose of estimating functional residual capacity by a closed spirometer circuit technic using a thermal conductivity meter for the analysis of helium in a procedure much like McMichael's version of the hydrogen method [*2*]. Subsequently closed helium methods were compared with various procedures by us [*3*] and by others [*4*]. Fowler [*5*] concludes that in comparison with other methods in common use "greater precision appears possible with closed circuit methods using serial helium or nitrogen analyses."

As described originally our method required a rather elaborate spirometer circuit and employed the technic introduced by Herrald and McMichael [*6*] to avoid errors believed to be attributable to the "oxygen storage effect" of Lassen, Cournand and Richards [*7,8*]. One of the valid criticisms of the earlier method was the complexity of the equipment [*5*].

We have re-examined some of the theoretical and practical considerations in estimating functional residual capacity by the dilution of helium and devised a greatly simplified version of the procedure which yields highly reproducible results with an instrument which is useful for a variety of other purposes and not unduly expensive.

Apparatus. A 9 L. respirometer§ was modified slightly (Fig. 1) by obliteration of dead space under the soda lime canister, substitution of a motor driven blower for the internal Sadd flutter valves, introduction of a water level gauge on the spirometer water jacket, provision of a shunt tube to pass some of the gas in the inspiratory tube through the helium meter analytical cell,‖ provision of stopcocks for rinsing tubing and charging the spirometer with helium and oxygen. The entire equipment as well as the small oxygen and helium tanks, the power supply and the helium indicator was mounted on an adjustable hospital bedside table. (Fig. 2.) This apparatus is easy to adjust to the patient whether he is in a prone, sitting or standing position, and constructed so that gas volumes in the spirometer bell may be measured with an accuracy within 10 ml., eliminating the need for a separate gas measuring burette for the helium charge. These modifications in no way impair the usefulness of the spirometer for other purposes. The substitution of the blower reduces resistance materially.¶

Determination of Dead Space. The blower is turned on, the spirometer is well rinsed with room air, the helium meter "warmed up" and balanced according to instructions, the water

§ Warren E. Collins Company, 555 Huntington Avenue, Boston, Massachusetts.

‖ Cambridge Instrument Company, Graybar Building, 420 Lexington Avenue, New York 17, New York. Meter calibrated to indicate 0 to 15 per cent helium in air saturated with water vapor.

¶ A commercial version of this equipment is available through either the Warren E. Collins Company or The Cambridge Instrument Co.

* From the Department of Medicine, Vanderbilt University School of Medicine and The Research Laboratory, Thayer Veterans Administration Hospital, Nashville, Tennessee, and The Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York. This investigation was supported in part through grants-in-aid for research from the Commonwealth Fund and the American College of Chest Physicians.

† Present address: Veterans Administration Center, Wood, Wisconsin.
‡ Present address: Newell Clinic, Chattanooga, Tennessee.

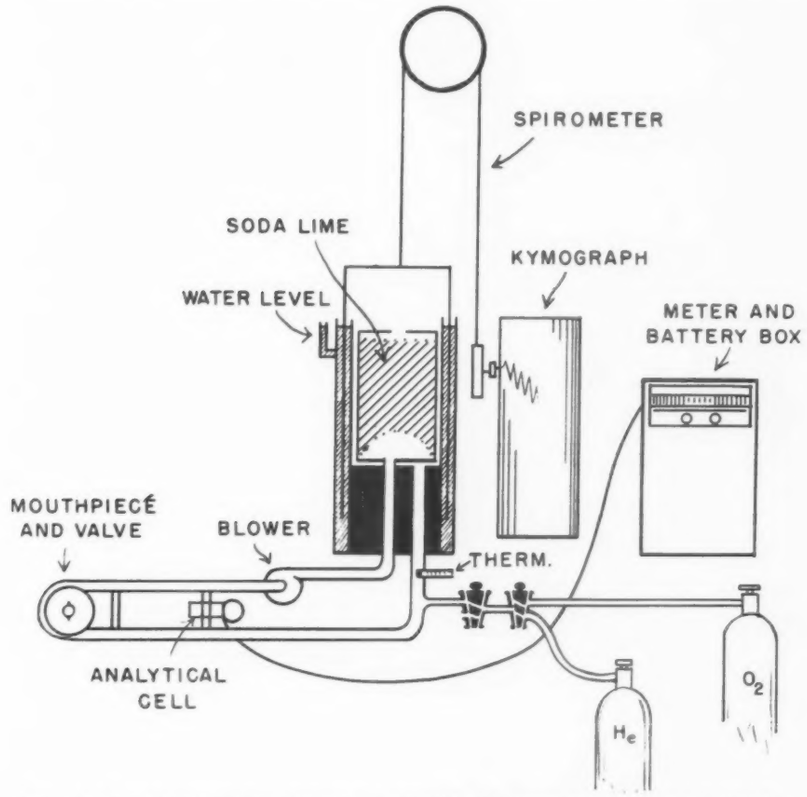


FIG. 1. Closed spirometer circuit for simplified helium lung volume method.

level of the spirometer accurately adjusted to the mark and the entire system carefully checked for leaks. The bell is pressed gently down to its zero position and the mouth piece valve closed to isolate the circuit from the room. The spirometer is tapped to eliminate backlash in the pulley or counter-weight chain and the pen tipped to the paper where a short line is inscribed by turning the kymograph drum by hand. The tubing from the helium tank is rinsed by flushing and approximately 800 ml. of helium is carefully admitted to the circuit. The spirometer is again tapped to eliminate backlash and a second short line inscribed on the paper. The circuit contents are mixed by the blower until a constant concentration of helium is indicated. The distance between the two lines inscribed on the kymograph paper is measured with a millimeter ruler of good quality and multiplied by the bell factor. The calculation* is simple:

$$\text{Dead space} = \left(\frac{\text{He added}}{\text{Final He Concentration}}\right) - \text{He added}$$

* Implicit is the assumption that the gas added is all helium and the meter is correctly calibrated. Both points are discussed in our earlier papers [7].

Analysis of Estimation of Dead Space, Helium Analytical and Spirometer Volume Errors. The mean of fifteen successive estimations of dead space was 5,540 ml. with a standard error of ±21 ml. (Table I.) The standard deviation was ±80.1 ml., giving a coefficient of variation of 1.4 per cent. The "error" of a single estimation exceeds 172 ml. in only 5 per cent of the instances. If the variations observed were attributable equally* to variations in helium analy-

* Dead space and functional residual capacity are measured quantities obtained by computation involving several variables. The error in such a quantity will depend of course on the errors in the component variables but also upon the ability of these variables to influence the final result. If R, the result, is a function of several variables

$$R = \phi(x,y,x \cdots)$$

and e_x is the error in x, e_y the error in y etc. then E, the error in R due to errors in x, will be e_x times the change in the function per unit change in x,

$$E_x = e_x \frac{d\phi(x,y,x \cdots)}{dx}$$

So also for E_y, E_z etc. the error in the final result due to the combined effect of errors in the component quantities is

$$E_R = \sqrt{(E_x)^2 + (E_y)^2 + (E_z)^2 \cdots}$$

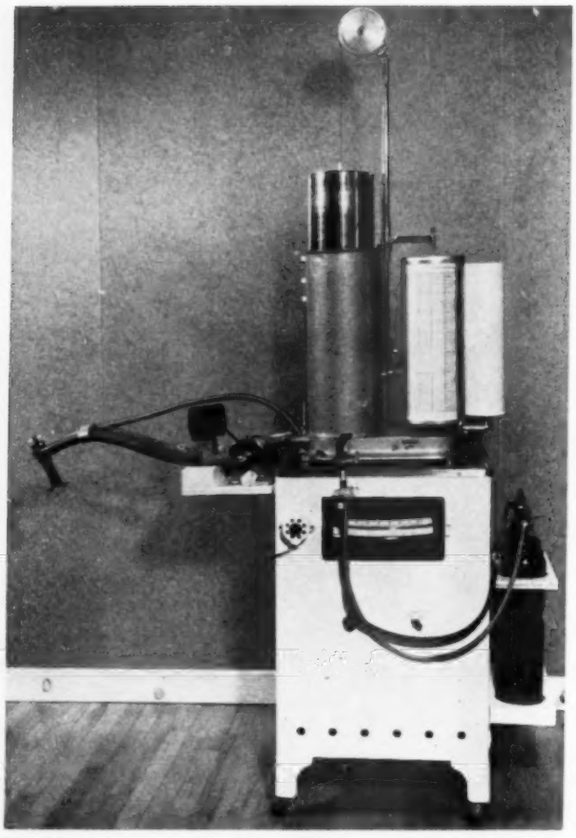


FIG. 2. Helium lung volume apparatus mounted on hospital bedside table.

sis and variations in spirometer volume measurement, the standard error for helium analysis would be ±0.08 volumes per cent and the standard error for spirometer measurement by the technic described would be ±5.1 ml. (Table II.) A similar meter tested frequently during three months with the same tank of 10 per cent helium exhibited a standard error of ±0.07 volumes per cent [*3*]. Variations in the analysis of helium and spirometer volume measurements of this magnitude would result in variations of less than 100 ml. in functional residual capacity in 95 per cent of the instances.*

* Dr. Lewis H. Bosher, Jr. in a personal communication contributes a valuable method for confirming the volumetric accuracy of the apparatus including the calibration of the analyzer. After determining the instrumental dead space a gas tight can of known volume is introduced into the spirometer circuit and the now enlarged "dead space" is again determined. In the instrument in use in this laboratory addition of a 650 ml. can varied the new dead space by ±20 ml. Another method is to admit increments of air through the mouth piece valve, checking volume on the paper.

TABLE I
FIFTEEN SERIAL ESTIMATIONS OF DEAD SPACE

Mean and standard error	5,540 ± 21 ml.
Standard deviation	80.1 ml.
"Error" of single estimation (P = 0.05)	172 ml.
Coefficient of variation	1.4%

Estimation of Functional Residual Capacity. The circuit is prepared as for the estimation of dead space, but more helium is admitted, about 1 L. for a normal person, and perhaps 1.5 L. for an emphysematous patient. This reduces error because the meter error is about the same at all readings, therefore proportionately less at high helium concentration. Final concentration of helium will be relatively large and since the analytical error is not proportional to the concentration but about the same over the whole scale of the indicator, the proportional error will be smaller at the higher concentrations of helium. The volume of helium added is measured with a good millimeter ruler, not by reference to calibrations on the kymograph paper. The measurement is more conveniently made after the paper is removed from the drum. After charging with helium the drum is turned by hand with the pen in a writing position to inscribe a line referred to as the "He line." (Fig. 3.) This is the volume at which the spirometer circuit initially contained a mixture of helium in air. The drum is returned to the starting position and enough oxygen admitted to provide for seven to ten minutes consumption of oxygen. The instrument is connected to the subject* who is permitted to rebreathe. As the respiratory base line climbs toward the He line the current through the meter is repeatedly readjusted because it drifts with the changing concentration of helium. The final helium concentration is read from the indicator at the time the respiratory base line crosses the He line. This is an easily located volumetric reference point at which time lung and spirometer circuit contain a mixture of gas approximately that of helium in air saturated with water vapor which is the circumstance in which the meter

* The mouth piece valve supplied with this instrument is excellent in many respects but has one notable failing. There is a position of the stopcock which permits intercommunication in all directions at once: to mouth piece, to room air and to spirometer circuit. It is difficult to make a valve without this defect which is not unduly bulky. If the valve is operated quickly, however, leakage is negligible.

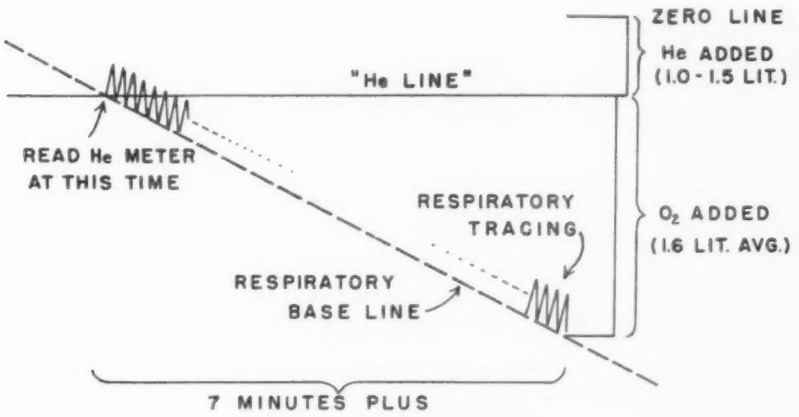


FIG. 3. Kymograph tracing.

TABLE II

HELIUM ANALYSIS AND SPIROMETRIC VOLUME MEASUREMENT
Based on Estimation of Dead Space

Standard error, He analysis*	±0.08 vol. %
Standard error, spiro volume measurement	+5.1 ml.

NOTE: With P = 0.05, variation in functional residual capacity of about 100 ml. would result from such "errors."

* S. E. for He by repeated analysis of same tank of 10 per cent He in air was ±0.07.

TABLE III

REPRODUCIBILITY OF THE FUNCTIONAL RESIDUAL CAPACITY

Functional Residual Capacity*	Difference Should Not Exceed "Error" of Mean (ml.)
All values (n = 42, σ = 121 ml.)	245 ± 86
Less than 5 L. (n = 29, σ = 75 ml.)	153 ± 54
More than 5 L. (n = 13, σ = 176 ml.)	385 ± 136

* Based on difference between first and second of forty-two serial duplicate estimations with P = 0.05.

was calibrated to indicate the concentration of helium correctly. The calculation* is also simple:

$$\text{Functional Residual Capacity} = \left(\frac{\text{He added}}{\text{Final He Concentration}}\right) - (\text{He added} + \text{dead space})$$

Reproducibility of the Functional Residual Capacity. Forty-two serial duplicate estimates of functional residual capacity were analyzed by taking the difference between the first and second as a deviation. (Table III.) For all values of functional residual capacity the standard deviation was ±121 ml. In 95 per cent of instances the difference between duplicates should not exceed 245 ml. The "error" of the mean of a pair is less than ±86 ml. in 95 per cent of the instances. Twenty-nine of these subjects and patients had a functional residual capacity less than 5 L. Among them, the variation was smaller with a standard deviation of only ±75 ml. In this case the difference between duplicates should not exceed 153 ml. and the error of the mean would be less than ±54 ml. in 95 per cent of the instances. Among the thirteen patients with advanced emphysema and functional residual capacity in excess of 5 L. the variation was larger with a standard deviation of ±176 ml. Duplicate estimates should differ by less than 385 ml. and the error of the mean of a pair would be less than ±136 ml. in 95 per cent of the instances.

Nitrogen Increment. Just before the spirometer circuit is connected to the subject the lung contains about 80 per cent nitrogen while the spirometer contains 5,540 ml. of air, 79.1 per cent of which is nitrogen, about 1 to 1.5 L. of helium and about 1.6 L. of added oxygen. Upon rebreathing, a redistribution of gas takes place with helium moving from spirometer to lung and nitrogen from lung to spirometer. This decrease in helium in the spirometer results in a relative increase of the concentration of nitrogen in the gas mixture in the spirometer so that the

* This volume must be corrected to the observed baseline in the respiratory tracing, otherwise errors as large as the tidal volume or more may result.

TABLE IV
THERMAL CONDUCTIVITY*

Air	5.68×10^{-5}
Nitrogen	5.24
Oxygen	5.63
Helium	33.9

* Calories/second/sq. cm. for a thickness of 1 cm. and a temperature difference of 1°C.

"non-helium" gas in the spirometer is not exactly of the composition of air by the time all the added oxygen has been consumed. At this moment samples from the circuit at the meter contain about 3 per cent more nitrogen than would a true mixture of helium in air.

This disturbance would be of no consequence were the helium analyzer not slightly sensitive to nitrogen which has a thermal conductivity a little lower than that of air. (Table IV.) The final volume (dead space + helium added + functional residual capacity) is about 10 L. In this system, 100 ml. volume changes of oxygen (i.e., 1 per cent concentration changes) caused differences in meter reading which would produce an apparent change of only 25 ml. in functional residual capacity due to corresponding changes in concentration of nitrogen (see footnote p. 825). This change is systematic, at least to the extent that it is always in the same direction. An increment of 3 per cent such as observed would be reflected by an apparent increase in functional residual capacity of 75 ml. Thus volumetric precision is not at all critical in relation to the exact instant the respiratory base line crosses the helium line since several hundred milliliter volume changes in O_2 in the circuit would produce only a few milliliters change in the functional residual capacity. Further, such errors are random and are reduced by replication. Steps could be taken to refine these adjustments. Our reasons for not doing so are discussed later.

Alveolar Inert Gas Related to Spirometer Inert Gas while Breathing Decreasing Oxygen Concentrations. It is generally accepted that samples of Haldane-Priestly alveolar air are reasonably representative of the average composition of the gas contained in the functional residual capacity [5] although it is now well known that there is much variation in composition from area to area within the lung and during different phases of the respiratory cycle.

In thirty-six studies the concentration of inert gas* in samples of alveolar air, first after breathing air and second after seven minutes of breathing from a spirometer circuit with decreasing oxygen, was determined. In the latter case the inert gas concentration of the final spirometer mixture was also determined. These observations were analyzed statistically (Table V) and are in accord with those which led to the "oxygen storage" concept [7,8] sometimes called "nitrogen lag" [4].

Inspection of the individual values led to a closer scrutiny of variance in the individual subject and among the two groups. The objections of the oxygen storage effect to the Christie method [9] relate to the following assumptions tacit in the Christie calculation. Alveolar inert gas during the breathing of air has the same relation to air inert gas that alveolar inert gas during breathing from spirometer mixtures with decreasing oxygen concentration has to spirometer inert gas, since one assumes 79.1 per cent for the inert gas concentration in the lung at the beginning of the run and spirometer inert gas at the end for concentration of inert gas in the lung at the end. Christie [9] was well aware of the possibility of error from these assumptions.

We investigated variance in this relation in two ways. First, the differences between the concentration of alveolar inert gas and its concentration in the breathing mixture were determined after breathing air and after breathing from decreasing spirometer oxygen concentrations. (Table V.) In either case after breathing from decreasing oxygen concentration the variance for the determination of the individual subject is larger than the difference which one seeks to correct. Variance in the relation of alveolar inert gas concentration breathing air to the inert gas concentration of air and variance in the relation of alveolar inert gas concentration to spirometer gas concentration after breathing from decreasing oxygen must be combined in evaluating the oxygen storage concept criticism of methods for functional residual capacity which involve breathing from decreasing oxygen (see footnote p. 824). The combined variance for the normal subjects is 1.02 per cent and the uncertainty ± 2.11 per cent (for $n = 20$ and $P = 0.05$), while among the emphysematous patients the combined variance is 2.53 per cent and the uncertainty ± 3.32 per cent (for $n = 16$ and $P =$

* The standard error of the estimation of inert gas was ± 0.103 volumes per cent (based on statistical analysis of over 100 serial duplicate determinations).

TABLE V

ALVEOLAR INERT GAS CONCENTRATION BREATHING AIR COMPARED WITH ALVEOLAR INERT GAS CONCENTRATION BREATHING FROM DECREASING SPIROMETRIC O_2 CONCENTRATION

Comparison of Two Methods

Data	Subjects*	
	Normal (n = 20) (%)	Emphysematous (n = 16) (%)
Differences Between Alveolar and Breathing Mixture Inert Gas		
Alveolar inert gas, after breathing air (mean)	80.2	81.5
Mean of differences (alveolar −79.1)	+1.08	+2.40
Standard deviation of the differences	±0.63	±0.56
Mean of differences (alveolar −spiro) with decreasing spiro O_2	+0.01	+2.55
Standard deviation of the differences	±0.73	±1.49
Ratios Between Alveolar and Breathing Mixture Inert Gas		
Ratio $\frac{\text{alveolar}}{79.1}$ after breathing air (mean)	1.014	1.030
Standard deviation of the ratios	±0.014	±0.025
Ratio $\frac{\text{alveolar}}{\text{spiro}}$ after breathing decreasing O_2 (mean)	1.002	1.048
Standard deviation of the ratios	±0.017	±0.108
$\frac{\text{Alveolar}}{79.1} - \frac{\text{alveolar}}{\text{spiro}}$ individual differences (mean)	+0.012	−0.018
Standard deviation of the differences	±0.017	±0.030

* Haldane-Priestly samples of average alveolar inert concentration of gas.

0.05). The second method involved analysis of variance in the ratio of alveolar inert gas concentration to the breathing mixture of inert gas concentration after breathing air and after breathing from decreasing oxygen. (Table v.)

The reasoning which led to the oxygen storage concept is above reproach, and the data presented in Table v confirm it for the normal subjects when one examines the mean of twenty determinations. The standard error of the mean differences is quite small. The difference in the relation of alveolar inert gas concentration to the breathing mixture is highly significant after breathing air as compared to breathing from decreasing oxygen. Nevertheless, the variances in sampling and analysis, and variation from person to person as demonstrated in Table v is sufficiently large so that there is no justification for elaborate instrumental and analytical maneuvers to eliminate errors attributed to breathing from decreasing oxygen concentrations because the uncertainties in the procedure designed to correct the errors are larger than the errors one seeks to correct.

Since in the calculation of functional residual capacity only the relation of final spirometer inert gas concentration to alveolar inert gas concentration is involved, and since in normal subjects these inert gas concentrations do not differ, there is little room for error due to "inert gas differences" or oxygen storage effect difficulties. Further, the mobility and diffusibility of helium should certainly achieve even more uniform intrapulmonary mixing than nitrogen.

Corrections. Application of any correction factor to an observation should be viewed with skepticism. As pointed out before [*3*], such factors may be responsible for small systematic differences between results obtained by different methods. Some helium must be absorbed by the body during rebreathing as previously discussed [*3*]. With higher concentrations of

TABLE VI
CORRECTIONS

Respiratory quotient	
Respiratory quotient = 0.8 and functional residual capacity 2 L.	−0.030 L.
He absorbed	
5 ml. absorbed and functional residual capacity + spiro volume − 10 L.	−0.100 L.
N_2 increment in spiro	
Average three per cent	−0.075 L.
Rounded total.	−0.2 L.

TABLE VII
EFFECT OF AN "ERROR" ON RATIO OF RESIDUAL TO TOTAL CAPACITY

Data	
In a normal subject with functional residual capacity = 0.8 L. and vital capacity = 3.6 L. an "error" of 200 ml. in functional residual capacity would change the ratio from 25 to 28 per cent.	+3.0%
In a patient with emphysema with functional residual capacity = 5 L.; expiratory reserve volume = 1.2 L. and vital capacity = 2.55 L. An "error" of 400 ml. in functional residual capacity would change the ratio from 60 to 62.5 per cent.	+2.5%

helium and large volumes in the circuit it is reasonable to assume that 15 ml. might be absorbed. The functional residual capacity would then appear to be 100 ml. larger than actually is the case. The correction for respiratory quotient is of highly dubious validity but it is small, only 30 ml. Nitrogen increment is responsible for errors in meter reading which result in apparent volume increases of about 75 ml. as shown. The rounded total of these three, 0.2 L., is deducted from the calculated functional residual capacity. (Table VI.) Corrections of temperature and water vapor are conveniently made from Comroe's table [5].

The Significance of Measurements of Functional Residual Capacity. The other fractions of the total pulmonary capacity are determined by direct spirometry. Fowler has reviewed testing procedures and accumulated normal standards for all fractions [5]. When the fractions are combined with an estimate of functional residual capacity one has data of two sorts: first, the estimated volume of the fractions, i.e., total capacity, residual volume, expiratory reserve volume, functional residual capacity, inspiratory capacity and vital capacity, and second, the ratio of one of these volumes to another, that is, of residual volume to total capacity, usually given as a per cent.

The normal standards to which observed pulmonary volumes may be referred have a coefficient of variation admitted to be from 10 to 25 per cent [5]. The variation in habitus from person to person and, more important, variation in the position of the diaphragm make it unlikely that normal values for total lung volume and its fractions will ever be predicted in any simple way with great accuracy. The volumetric data are therefore most useful in the same person from time to time.

The ratio of residual volume to total capacity, however, is less variable. Despite inconsistencies in intrapulmonary gas mixing and other valid criticisms of the procedure, the ratio of residual volume to total capacity remains the best way to characterize numerically the degree of pulmonary distention which may exist at the moment. There are a variety of criteria for interpreting the percentile ratio of residual volume to total capacity. Traditionally the "upper limit of normal" is given as 35 per cent for men and 40 per cent for women. For those under fifty years of age this is almost certainly too high. A simple expression which embraces virtually all of the published data for males is that the mean ratio per cent of residual volume to total capacity is 20 per cent from childhood to forty years of age and thereafter is equal to one-half the age of the patient. The range of normal is ±8, i.e., from 12 to 28 per cent until forty years of age and increasing thereafter to ±16, i.e., from 24 to 56 per cent at eighty years of age. The data are sparse in the older age groups. Women tend to higher values, perhaps by 5 per cent, but adequate data are lacking.

Although the reproducibility of the simple method described in this report is excellent, there may be inherent systematic errors. It is worthwhile, then, to consider what effect moderate errors would have on the ratio of residual volume to total capacity. An error of 200 ml. in functional residual capacity in a normal subject would change the ratio of residual volume to total capacity about 3 per cent; and in severe emphysema, a 400 ml. error would change it slightly less. (Table VII.) This would hardly alter the interpretation of the findings in an individual subject. In the same patient from time to time, systematic errors

would not at all affect changes which might be observed and the reproducibility of the method is the important consideration.

SUMMARY AND CONCLUSION

Twenty-seven years ago Christie was able to review "no less than forty-seven papers dealing with different methods available up to that time for measuring residual air indirectly" [*9*]. From the data presented here and the work of others referred to, it appears likely that an undue amount of effort has been devoted to refining methodology without corresponding compensation in the quality of results but with the very definite effect of making the estimation of lung volumes a more laborious affair, thus deterring many from the use of this valuable clinical tool. A simple rapid version of the helium method for estimating functional residual capacity is described employing equipment which is not unduly expensive and which is useful for a variety of other tests of pulmonary function. Results are highly reproducible. Evidence that the method is not subject to serious systematic error is presented.

REFERENCES

1. Meneely, G. R. and Kaltreider, N. L. Use of helium for determination of pulmonary capacity. *Proc. Soc. Exper. Biol. & Med.*, 46: 266, 1941.
2. McMichael, J. A rapid method of determining lung capacity. *Clin. Sc.*, 4: 167, 1939.
3. Meneely, G. R. and Kaltreider, N. L. The volume of the lung determined by helium dilution. Description of the method and comparison with other procedures. *J. Clin. Invest.*, 28: 129, 1949.
4. Gilson, J. C. and Hugh-Jones, P. The measurement of the total lung volume and breathing capacity. *Clin. Sc.*, 7: 185, 1949.
5. Fowler, W. S. Specific tests of pulmonary function. A. Lung volumes. In: Methods in Medical Research, vol. 2, p. 181. Edited by Comroe, J. H., Jr. Chicago, 1950. The Year Book Publishers.
6. Herrald, F. J. C. and McMichael, J. Determination of lung volume: Simple constant volume modification of Christie's method. *Proc. Roy. Soc. London, s. B.*, 126: 491, 1939.
7. Lassen, H. C. A., Cournand, A. and Richards, D. W., Jr. Distribution of respiratory gases in closed breathing circuit. I. In normal subjects. *J. Clin. Invest.*, 16: 1, 1937.
8. Cournand, A., Lassen, H. C. A. and Richards, D. W., Jr. Distribution of respiratory gases in closed breathing circuit. II. Pulmonary fibrosis and emphysema. *J. Clin. Invest.*, 16: 9, 1937.
9. Christie, R. V. The lung volume and its subdivisions *J. Clin. Invest.*, 11: 1099, 1932.

Mucormycosis*

Two Cases of Disseminated Disease with Cultural Identification of Rhizopus; Review of Literature

RAYMOND A. MCBRIDE, M.D., JOSEPH M. CORSON, M.D. *and* GUSTAVE J. DAMMIN, M.D.
Boston, Massachusetts

REPORTS of disease in man due to or complicated by fungi of the order mucorales (mucormycosis) are appearing with increasing frequency in the medical literature. In most of the cases reported mucormycosis has been considered to be a complicating infection in a patient debilitated by another disease and/or receiving antibiotic, steroid or radiation therapy. The relationship between mucormycosis and the uncontrolled diabetic state has been stressed, and many experiments have been performed with the aim of elucidating the factors in the experimental diabetic state which favor the uncontrolled growth of these ordinarily saprophytic organisms.

Two instances of mucormycosis have been identified recently at autopsy at the Peter Bent Brigham Hospital. In the first case septic abortion was complicated by renal cortical necrosis, anuria and uremia, and by disseminated mucormycosis. In the second case hepatic dysfunction was complicated by acute renal tubular necrosis and uremia. Disseminated mucormycosis, which was discovered at autopsy, may have been the cause of the hepatic lesion. Because of the few instances in which cultural identification of the fungus in human mucormycosis has been reported (the diagnosis usually being based on the morphology of the fungus in tissues) the isolation of Rhizopus arrhizus in the second case was of particular interest.

It is our purpose to discuss the clinical and pathological features of these two cases and to summarize the current concepts of pathogenesis, diagnosis and treatment of this usually fatal infection.

CASE REPORTS

CASE 1. E. F. (PBBH No. 5L122), a thirty-two year old white woman, was admitted to the Peter Bent Brigham Hospital on July 6, 1957, with anuria and uremia. One week prior to admission she was alleged to have attempted to induce an abortion of an estimated two and a half months pregnancy by insertion of a rubber catheter into the uterus. The next day she had fever and abdominal pain. She was given penicillin and sulfonamides, and was admitted to another hospital because of anuria and a temperature of 104°F. The hemoglobin was 13 gm. per cent, white blood cells, 27,000 per cu. mm. and serum non-protein nitrogen 170 mg. per cent. Escherichia coli was identified in the vaginal discharge. She was treated with penicillin, tetracycline and intravenous fluids, and was then transferred to the Peter Bent Brigham Hospital. On admission she was severely ill and comatose. The blood pressure was 90/50 mm. Hg, temperature 98.6°F., pulse 108 per minute and respirations irregular and gasping at 28 per minute. There were petechiae and ecchymoses over the skin. Rales were heard in the inferior half of each lung. The abdomen was soft, tympanitic and diffusely tender. The liver was palpable 2 cm. below the right costal margin. The abdominal reflexes were absent. The hematocrit was 34 per cent, white blood cells 53,000 per cu. mm. with 92 per cent neutrophils, erythrocyte sedimentation rate (Wintrobe) 23 mm. in one hour, blood urea nitrogen 200 mg. per cent, blood sugar 146 mg. per cent, sodium 131 mEq./L., potassium 6.8 mEq./L. and chlorides 87 mEq./L. Blood cultures showed no growth at ten days. During dialysis with the artificial

* From the Department of Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts. This study was supported in part by a Pathology Research Training Grant (HTS 5274) from the National Heart Institute.

kidney, which was begun soon after admission, the patient had a generalized myoclonic convulsion and died.

At autopsy (PBBH A 57-225X) there were ecchymoses over the trunk and extremities, and petechiae over the chest and nose. The peritoneal cavity contained 200 ml. of serosanguineous fluid. Patchy wedges of terminal ileum were black and necrotic. (Fig. 1.) The mesentery adjacent to these areas was thickened, dark red and indurated. There were numerous petechiae in the epicardial fat and beneath the endocardium of the left ventricle. Large areas of the mucosa of the larynx, of the pyriform recesses and of the hypopharynx were black and ulcerated. The mucosa of the trachea and large bronchi was injected and the lumen contained black gastric contents.

The right lung weighed 580 gm., the left, 500 gm. Scattered over the pleural surface of all lobes and extending into the underlying parenchyma were numerous firm, red, wedge-shaped infarcts measuring up to 3 cm. Small vessels adjacent to the infarcts were occluded by gray thrombi. The cut surface of the lower lobes was red and hypocrepitant.

The spleen weighed 180 gm. and was red and firm. Scattered beneath the capsule there were many small purple infarcts. The adrenals together weighed 35 gm. and were hemorrhagic and firm. Medullary veins were occluded by thrombi. The kidneys together weighed 425 gm. and were swollen and pale. The cortices were studded with small, subcapsular yellow infarcts.

The uterus was enlarged to 12 by 8 by 4 cm. The cervix was soft and eroded. A large plaque of friable, red decidua adhered to the endometrium. The myometrium and serosa were normal.

The mucosa of the distal third of the esophagus was black and ulcerated. (Fig. 2.) Within the distal 40 cm. of the ileum, there were numerous dark red infarcts. The adjacent mesentery was red and indurated.

The meninges were normal. There were hemorrhages in the pons, midbrain, medulla oblongata and cerebellum.

On microscopic examination it was found that many tissues were invaded by large, branching, non-septate fungal hyphae which measured approximately 10 to 20 μ. in diameter and up to 250 μ. in length. The hyphae, which were PAS-positive and readily seen in sections stained with hematoxylin and eosin, were, on the basis of their morphology, considered to belong to the order mucorales.

The mucosa of the larynx was ulcerated and the mucosa and submucosa were invaded by hyphae, some of which penetrated the walls of the blood vessels. An intense inflammatory reaction with necrosis was present about the hyphae. In the lung, hyphae were found in scant numbers in the lumens of bronchi and in abundance in adjacent blood vessels where,

FIG. 1. Case 1. Multiple segments of dark necrotic ileum and intervening viable intestine. Note the mesenteric necrosis adjacent to the segments of involved bowel.

enmeshed in sheets of neutrophils, they occluded the lumens. Hyphae extended through vessel walls into contiguous alveoli. (Fig. 3.) Many of the small blood vessels not containing hyphae were occluded by fibrin-like thrombi. In patchy areas of the parenchyma there was extensive hemorrhage, edema, neutrophilic leukocytic infiltration and necrosis of the alveolar walls.

Fibrin-like thrombi were found in hepatic sinusoids, adrenal sinusoids and medullary veins, splenic vessels, renal arterioles and glomerular tufts, ovarian veins

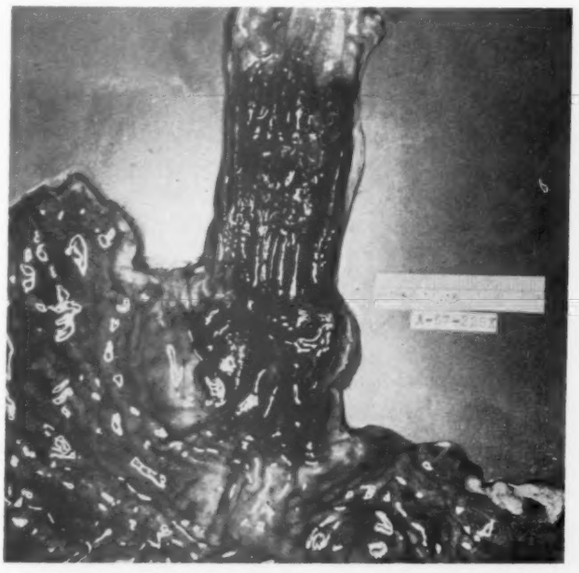


FIG. 2. Case 1. Necrotic membranous inflammation of the distal esophagus with sharp separation from uninvolved esophagus (above) and stomach (below).

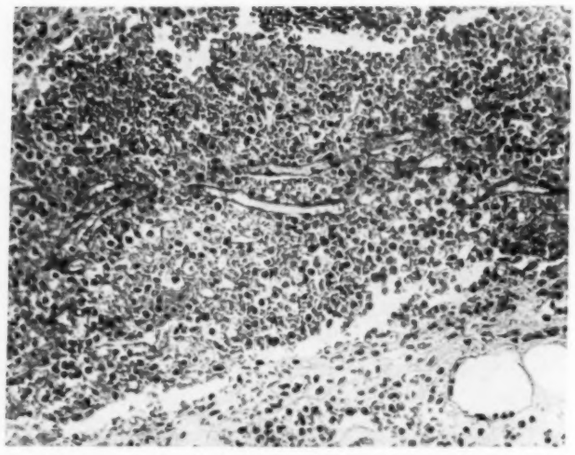


FIG. 3. Case I. Typical large non-septate hyphae within a pulmonary alveolus. Note the extensive hemorrhage and the cellular reaction consisting of both small round cells and neutrophiles. Hematoxylin and eosin, original magnification × 286.

and in vessels of the myometrium. There was extensive hemorrhage and necrosis of the adrenals and focal subcapsular hemorrhage of the spleen and kidneys. Tubules of the renal medulla contained brown, granular casts and their lining epithelium was focally necrotic.

Necrotic decidua infiltrated by neutrophils and erythrocytes was attached to the endometrium. The left ovary contained a corpus luteum of pregnancy and both ovaries contained small zones of hemorrhage.

In the pharynx, massive necrosis and hemorrhage extended from the mucosa deep into the skeletal muscle. Hyphae were present in all layers, often surrounded by pools of neutrophils. In the wall of the distal segment of the esophagus and in large segments of the distal ileum, there were many hyphae and extensive areas of necrosis, hemorrhage, neutrophilic infiltration and vascular thrombosis. Mesenteric arteries and veins adjacent to the ileum were plugged by masses of fungal hyphae.

There were areas of recent hemorrhage in the pons, cerebellum, medulla oblongata and midbrain, but no hyphae were found. No pathologic changes were seen in the pituitary gland.

Staphylococcus aureus was cultured from the lung and E. coli from the uterus.

Anatomic diagnoses: Necrotic decidua consistent with interrupted pregnancy; multiple intravascular fibrin-like thrombi in the lungs, liver, spleen, adrenals, kidneys, uterus and ovaries; hemorrhagic cortical necrosis of the kidneys, adrenals and spleen; mucormycosis of the lungs and gastrointestinal tract with (1) vascular invasion by the fungi with occlusion of pulmonary, mesenteric and esophageal vessels, (2) necrotizing pharyngitis, laryngitis and esophagitis and (3) hemorrhagic infarcts of the lungs and ileum.

CASE II. R. S. (PBBH No. 3M683), a twenty-six year old white man, was admitted to the Peter Bent Brigham Hospital on May 10, 1958, with fever, jaundice and abdominal pain. Thirteen days before admission he noted the onset of malaise, anorexia, nausea, vomiting, chilliness and fever. Ten days prior to admission he noted the precipitous onset of severe pain in the epigastrium and right upper quadrant of the abdomen together with shaking chills, nausea, vomiting and dark urine. He was admitted to another hospital with a temperature of 103°F., icterus and tenderness in the right upper quadrant of the abdomen. He was treated with Combiotic,® intravenous fluids, Compazine® and a single dose of cortisone. Severe nausea, vomiting and abdominal pain marked his hospital course. Anuria, hypertension, epistaxis and petechiae appeared and he was transferred to the Peter Bent Brigham Hospital.

On admission he was found to be a well developed, acutely ill young man. His chief complaint was pain in the upper abdominal quadrant. The blood pressure was 180/110 mm. Hg, temperature 98.4°F., pulse 100 and respirations 20 per minute. He was severely jaundiced and the face, neck, shoulders and upper part of the chest were edematous. The tongue was dry, red and swollen. The heart was enlarged to the left and a grade 2 apical systolic murmur was heard. The lungs were clear. The liver was firm, non-tender and palpable 1 cm. below the right costal margin. There was marked tenderness and muscle guarding in the right upper quadrant inferior to the liver. The abdomen was distended and tympanitic, the bowel sounds hypoactive.

Family history revealed that the patient's mother-in-law was jaundiced at the time of onset of his illness. He denied contact with hepatotoxins.

The urine was dark brown, positive for bile, negative for sugar and contained 2 plus-protein, occasional white cells and many tyrosine and leucine crystals. The hematocrit was 49.5 per cent, white blood cells 13,100 per cu. mm. with 81 per cent neutrophils and 5 per cent band forms. The platelets appeared normal. The icterus index was greater than 100, blood urea nitrogen 158 mg. per cent, blood sugar 152 mg. per cent, serum sodium 117 mEq./L., potassium 5.2 mEq./L., carbon dioxide 18.8 mM./L., chlorides 83 mEq./L., serum amylase 462 units, calcium 4.8 mEq./L., phosphorus 4 mM./L., alkaline phosphatase 4 Bodansky units, thymol turbidity 94 units, serum bilirubin 13.4 mg. per cent, direct, and 7.2 mg. per cent indirect, lactic dehydrogenase 340 units, uric acid 34.5 mg. per cent, total protein 6 gm. with a normal electrophoretic pattern except for a gamma

globulin of 1.5 gm. per cent. The reaction to the Hinton test for syphilis was negative. The prothrombin time was 56.5 per cent of normal, clotting time 22 minutes, tourniquet test negative, clot retraction good and fibrinolysins not demonstrable. The cerebrospinal fluid was normal except for xanthochromia. Electrocardiograms showed left ventricular hypertrophy and signs of hyperkalemia and hypocalcemia. Chest roentgenograms were within normal limits. Roentgenograms of the abdomen showed generalized gaseous distention of the large and small bowel. Retrograde pyelograms did not demonstrate obstruction of the urinary tract.

The patient was treated with Hykinone,® Berocca C,® Benadryl,® Compazine, chloramphenicol and was given approximately 500 ml. of intravenous 50 per cent dextrose in water daily. During the first few days he remained anuric and there was progressive increase in the serum potassium (7.6 mEq./L.), blood urea nitrogen (238 mg. per cent) and uric acid (37 mg. per cent). Persistent severe abdominal pain remained his major complaint. The serum bilirubin fell to 6 mg. per cent. The white blood cell count increased to 39,000 per cu. mm. On May 21, dialysis was performed with the artificial kidney, the serum potassium was reduced to 6.6 mEq./L. and the blood urea nitrogen to 144 mg. per cent. On May 22, exploratory laparotomy, cholecystostomy and liver biopsy were performed. No obstruction of the biliary tract or gall stones were found and the pancreas, peritoneum and adjacent bowel examined through the limited incision appeared normal. In the biopsy specimens of the liver and gallbladder, multifocal centrolobular hepatic necrosis of a toxic variety and chronic cholecystitis were identified. Postoperatively, there was rising fever, increase in amylase (over 1,000 units), recurrence of high blood urea nitrogen and hyperkalemia. Daily blood transfusions maintained the hematocrit at about 20 per cent. On May 25, dialysis was repeated. On this day the patient had severe pain and tenderness in the lower right quadrant without clinical evidence of peritonitis. Despite administration of streptomycin and compound F he became hypotensive and comatose. He passed a single guaiac-positive stool. Multiple cultures of the blood, urine, cerebrospinal fluid, ascitic fluid and gallbladder yielded no growth. He died in shock on May 28, 1958.

At autopsy (PBBH A 58-160) there was jaundice of the skin and sclerae. The peritoneal cavity contained 300 ml. of clear yellow fluid. Segmental areas of ileum, cecum and ascending colon were red-black and infarcted. The mesentery adjacent to these segments of bowel was thickened, focally hemorrhagic, covered with shreds of fibrin and studded with small yellow granular areas of necrosis. Large mesenteric vessels were patent; however, many of the smaller terminal arcuate branches adjacent to the areas of infarcted bowel were completely occluded by red-gray thrombi.

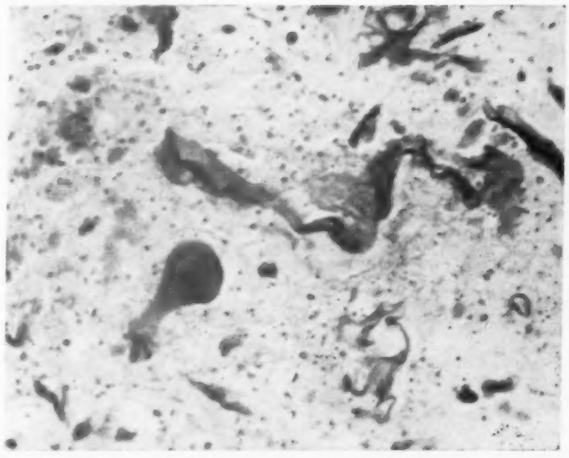


FIG. 4. Case II. Spore of R. arrhizus, together with hyphae, within a pulmonary alveolus. Periodic acid-Schiff stain original magnification × 840.

Except for a 3 by 2 cm. area of recent hemorrhage beneath the endocardium of the left ventricle the heart was normal. The right lung weighed 650 gm., the left 600 gm. Dependent and inferior portions of all lobes were subcrepitant and blue-gray, and on sectioning exuded copious pink, frothy fluid. In the lingula of the left lung there was a dark red, firm, wedge-shaped area measuring 5 by 3 by 3 cm. and in the anterior medial basal segment of the lower lobe of the left lung there was a similar although smaller area. Several small blood vessels adjacent to these firm foci were completely occluded by red-gray thrombi.

The liver weighed 2,000 gm. and was yellow, tense and friable, and centrolobular areas were bile-stained. The extrahepatic bile ducts were unobstructed. Each kidney weighed 200 gm. and was swollen, pale green and soft with a dark, red-brown medulla.

The mucosa of the distal half of the esophagus and of the gastric cardia was red, granular and covered with a shaggy green membrane. In the distal portion of the ileum there were areas of ulceration and necrosis, the largest of which involved the entire circumference of a 37 cm. segment of bowel. The ileocecal valve, cecum, appendix and proximal 9 cm. of ascending colon appeared infarcted. There was a 3 by 4 cm. mucosal ulcer 6 cm. proximal to the anorectal junction.

The brain appeared normal except for a 1.5 cm. area of fresh hemorrhage in the cerebellum.

On microscopic examination of the left lung there was extensive hemorrhage and necrosis, with occlusion of numerous blood vessels by recent thrombi.

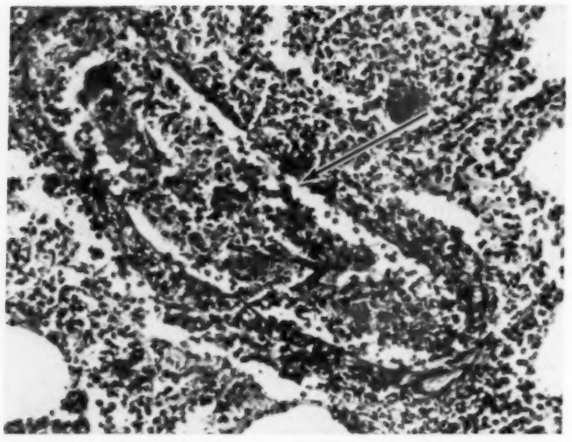


FIG. 5. Case II. A mesenteric blood vessel containing hyphae of R. arrhizus within the lumen and wall. Periodic acid-Schiff stain, original magnification × 386.

Within the lumens of these vessels, penetrating their walls and permeating the surrounding alveoli, were many large, branching, coenocytic, PAS-positive hyphae varying from about 5 to 15 μ. in diameter and measuring up to 200 μ. in length. In some of the alveoli and vessels invaded by the fungi there was little or no inflammatory reaction, but in others there was an intense neutrophilic infiltration. No fungi were seen in the bronchi. A single spore was found in an alveolus. (Fig. 4.)

Hepatic cells surrounding the central veins were necrotic and the sinusoids in these areas were dilated and filled with acidophilic material and a few neutrophils. Centrolobular bile canaliculi contained inspissated bile. In portal areas there was slight proliferation of the bile ducts and neutrophilic infiltration.

The renal cortical tubules were dilated, widely separated by interstitial edema, and contained birefringent, pale green crystals resembling leucine. Large, granular, red-brown pigment casts filled many of the cortical and medullary tubules. In and surrounding many of the medullary tubules, often adjacent to the pigment casts, were small collections of neutrophils, histiocytes and lymphocytes.

The epithelium of the lower third of the esophagus and of the gastric cardia was replaced by fibrinopurulent exudate. The lamina propria and muscularis mucosa were edematous and infiltrated by neutrophils. Hyphae similar to those in the lungs extended in profusion through vascular channels and the esophageal wall. In infarcted areas of the ileum, cecum and rectum the mucosa was ulcerated, the wall congested, edematous and in some regions extensively necrotic. Hyphae similar to those in the lungs penetrated the bowel wall and adjacent mesentery, resulting in vascular necrosis and thrombotic occlusion. (Fig. 5.) The pancreatic acini were dilated and filled with acidophilic material. The stroma was infiltrated by clusters of neutrophils and in a few foci stroma as well as parenchyma was necrotic.

An area of the cerebellum was disrupted by extravasated erythrocytes, but no fungi were present. Small areas of the pituitary were necrotic.

R. arrhizus was cultured from the mesentery of the small bowel.

Anatomic diagnoses: Mucormycosis of the lung and gastrointestinal tract with (1) vascular invasion by fungi and occlusion of mesenteric, esophageal and pulmonary blood vessels, (2) hemorrhagic infarcts of the ileum, cecum, appendix and left lung and (3) necrotizing esophagitis and proctitis; centrolobular hepatic necrosis with slight intracanicular bile statis; jaundice; ischemic nephrosis; acute, focal pancreatitis; acute and chronic cholecystitis (cholecystostomy tube in place).

COMMENT ON CASES PRESENTED

In the first case septic abortion was associated with multiple fibrin thrombi and renal cortical necrosis with renal failure. The manipulation of the uterine pregnancy presumably introduced coliform organisms, with death of the fetus and onset of shock as a result of liberation into the maternal circulation of placental toxins or of endotoxins produced by gram-negative organisms. As a probable consequence there was widespread intravascular deposition of fibrin followed by cortical necrosis of the kidneys and adrenals leading to uremia and adrenal insufficiency. Endotoxins related to intrauterine E. coli may have produced a generalized Shwartzman reaction as proposed by McKay et al. [*1*]. Mucormycosis appeared to be a late complication. Sheldon and Hertig [*2*] in a study of renal cortical necrosis related to toxic separation of the placenta noted cecal ulcers associated with intravascular fibrin thrombi in the bowel wall. In this patient with widespread intravascular fibrin deposition, erosions of the mucous membrane of the bowel may have occurred. Such defects in either the gastrointestinal or tracheobronchial mucosa could have become sites of entry of the fungus. Death was due to uremia with cerebral hemorrhage, disseminated mucormycosis and terminal aspiration pneumonia.

The nature of the liver disease in Case II (R. S.) is obscure. The limited cellular infiltration in the liver, among other factors, tended to rule out viral hepatitis as the etiologic agent.

Leptospirosis was considered; however the early appearance of the jaundice, the absence of persistent fever, the presence of focal hepatic necrosis without regeneration, and the absence of characteristic skeletal muscle or myocardial lesions argued against this diagnosis. Although no history of chemical or drug toxicity was obtained, the morphologic picture was most compatible with toxic hepatitis. Hepatitis, due to an unknown agent, may have occurred first, with mucormycosis superimposed late in the patient's illness; however, invasion of the gastrointestinal tract by the fungus possibly may have been the initial event and the cause of the hepatic alterations. The absence of occult blood in the stool until late in the illness, and the absence of chronic inflammatory cell infiltration and of fibroblastic proliferation in the areas of fungal proliferation suggest that massive invasion by the fungus was a late event.

Ischemic tubular necrosis developed early in the clinical course after prolonged vomiting, and reputed inadequate fluid replacement. Death was due to uremia and disseminated mucormycosis.

In the two cases reported herein, the diagnosis of mucormycosis was first made at autopsy on the basis of the characteristic morphology of the fungus in tissue sections. In the second case the causative organism was further identified as R. arrhizus [*3,4*] on morphological grounds after culture on Sabouraud's medium.

THE FUNGUS

The term "mucormycosis" refers to infection by a member of the order mucorales. These fungi belong to the class phycomycetes [*5*] and are commonly known as bread molds or sugar fungi. They are ubiquitous saprophytes requiring only sugar and simple carbon compounds as nutrients [*5,6*] and are frequently found on decaying vegetable matter or in the soil [*7,8*]. Mucormycosis in man has been due usually to members of one of three genera of the mucorales: mucor, absidia or rhizopus.

The mucorales grow rapidly on Sabouraud's medium, completely filling a petri dish in a few days. They are characterized by the presence of non-motile, asexual spores (sporangiospores) contained within a spore case (sporangium). (Figs. 6, 7 and 8.) Sexual spores (zygospores) are

FIG. 6. Case II. R. arrhizus, aerial mycelia with sporangiophores (arrow 2) bearing prominent spherical sporangia (arrow 1). Sabouraud's medium, unstained, original magnification × 110.

produced by the fusion of gametangia derived from the vegetative mycelium. Differentiation of the three genera causing mucormycosis in man is based on morphologic differences in sexual and asexual spores appearing after growth in suitable culture media and on the presence or absence of stolons (runners) and rhizoids (holdfasts) and their relation to fascicles (sporangio-

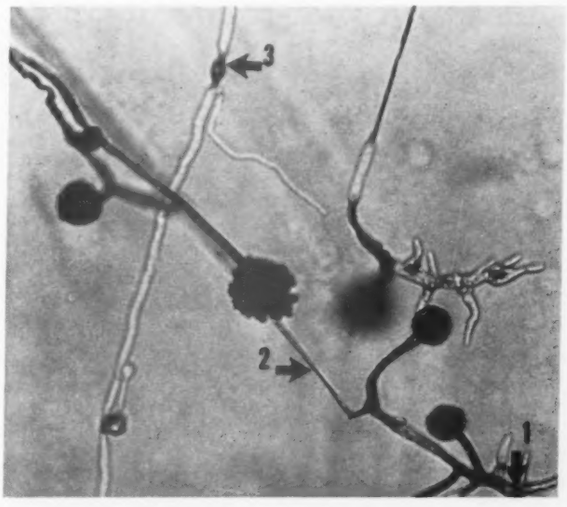


FIG. 7. Case II. R. arrhizus, aerial and vegetative mycelia with sporangia. Note the sporangiophores arising from stolons (arrow 2) opposite rhizoids (arrow 1). Arrow 3 indicates a zygospore at the point of fusion of two adjacent hyphae. Sabouraud's medium, unstained, original magnification × 180.

TABLE I

MUCORMYCOSIS IN MAN

Case No.	Author	Age (yr.), Race and Sex	Geographic Location	Predisposing Factors	Organs Involved by Mucor	Cultural Verification	Cause of Death when Fatal
1	Paltauf, 1885 [9]	52,W,M	Austria	Not stated	Intestine, lungs, brain	No	Mycotic septicemia
2	Gregory et al., 1943 [11]	43,N,F	Maryland	Diabetes with acidosis	Soft tissue of nose, eye, internal carotid artery, cavernous sinus, brain	No	Cerebral mucormycosis, diabetic acidosis
3	.....	52,N,F	Maryland	Diabetes with acidosis	Nose, ethmoid sinus, ocular muscles, brain	No	Cerebral mucormycosis, diabetic acidosis
4	.....	75,W,M	Maryland	Probable diabetes; cirrhosis of the liver	Eye, optic nerve, medulla oblongata, pons cervical spinal cord, meninges	No	Cerebral mucormycosis
5	LeCompte Meissner, 1947 [31]	57,W,M	Massachusetts	Diabetes with acidosis; hemochromatosis	Brain	No	Cerebral mucormycosis, diabetic acidosis
6	Moore et al., 1949 [32]	37,W,F	Wisconsin	Mucous colitis; peritonitis	Large bowel	No	Intestinal mucormycosis, peritonitis
7	Lloyd et al., 1949 [33]	26,N,F	Massachusetts	Diabetes; syphilis; pregnancy	Lung	No	Pulmonary mucormycosis
8	Wolf, Cowen, 1949 [34]	42,N,M	Not stated	Diabetes; glomerulosclerosis; central necrosis of the liver	Brain	No	Cerebral mucormycosis, azotemia
9	Stratemeier, 1950 [35]	32,N,F	Pennsylvania	Diabetes with acidosis	Anterior ethmoid, nose, brain, meninges	No	Diabetic acidosis, pneumonia, cerebral mucormycosis
10	Murphy, Bornstein, 1950 [36]	40,W,M	North Carolina	Two year old shot gun wound of the chest	External auditory meatus, subpleural pulmonary mass (excised)	Absidia italiana	Apparent recovery
11	Wadsworth, 1951 [37]	10,M	Not stated	Coats disease (exudative retinitis)	Eye (enucleated)	No	Apparent recovery
12	Martin et al., 1954 [38]	2½/12,W,M	Texas	Diarrhea; dehydration; acidosis; chemotherapy	Meninges, eye, skin of face, esophagus, pleura, lungs, pancreas, bone marrow	No	Widespread mucormycosis
13	Kurrein, 1954 [39]	5/12,M	England	Bloody diarrhea; anuria; glomerulitis; respiratory infection	Brain	No	Cerebral mucormycosis, uremia
14	Zimmerman, 1955 [13]	53,N,M	Not stated	Cirrhosis of the liver; multiple myeloma; therapy with urethane; cortisone, x-ray	Heart (coronary veins), lung, kidney	Not stated	Cardiac infarct secondary to mucorthrombosis of coronary veins
15	Bauer et al., 1955 [40]	25,W,M	Not stated	Diabetes; glomerulosclerosis; renal papillary necrosis; chronic bronchitis; penicillin therapy	Brain, meninges	No	Cerebral mucormycosis, diabetes
16	.....	40,N	Not stated	Diabetes with acidosis; acute renal tubular necrosis	Brain, internal carotid artery, nasal mucosa, ethmoid sinus	Rhizopus oryzae	Cerebral mucormycosis, acidosis, renal tubular necrosis
17	Gunson, Bowden, 1955 [41]	5,F	Ontario	Chronic glomerulonephritis; nephrotic syndrome; uremia; cortisone therapy	Brain with thrombosis of the internal carotid and middle cerebral arteries	No	Cerebral mucormycosis, uremia
18	Harris, 1955 [18]	14,N,F	North Carolina	Diabetes; respiratory infection	Eye (probably retro-orbital), soft palate, tongue, probable cranial nerves, internal jugular vein, nasal sinuses, cheek	Rhizopus arrhizus (palatal biopsy)	Recovery
19	Foushee, Beck, 1956 [42]	65,W,F	North Carolina	Possible diabetes; cholecystitis; cholelithiasis; carcinoma of the kidney; bacterial abscess of the liver; duodenal ulcer; status postoperative	Brain	No	Cerebral mucormycosis, infection (liver abscess)
20	Baker, 1956 [16]	47,W,M	North Carolina	Leukemia; ACTH, cortisone, antibiotic therapy	Lung	No	Pulmonary mucormycosis, leukemia
21	.....	23,W,M	North Carolina	Leukemia; 6-mercaptopurine, antibiotic therapy	Lung	No	Pulmonary mucormycosis, leukemia
22	.....	51,W,M	South Carolina	Diabetes	Lung	No	Pulmonary mucormycosis
23	.....	50,N,M	North Carolina	Diabetes with acidosis; pyelonephritis; uremia	Lung	No	Diabetic acidosis, renal insufficient, pulmonary mucormycosis
24	.....	3,N,F	North Carolina	Body burns; penicillin therapy	Lung, spleen, liver	No	Disseminated mucormycosis, body burns,
25	.....	3,W,M	Arizona	Not stated	Lung, kidney, liver, large vessels	No	Disseminated mucormycosis
26	Baker, 1948 [16,43]	3,W,F	Texas	Diabetes; pyelonephritis; lipid histiocytosis of the spleen, liver	Lung, brain, orbit	No	Orbital, cerebral, pulmonary mucormycosis
27	C.P.C., 1956 [4]	53,F	Australia	Diabetic acidosis; pneumonia	Lung, brain, eye	No	Disseminated mucormycosis
28	Keye, Magee, 1956 [45]	38	Missouri	Hodgkin's disease; tuberculosis	Lungs	No	Primary disease
29	.....	23	Missouri	Acute myelogenous leukemia	Lungs	No	Primary disease
30	.....	1½	Missouri	Acute lymphocytic leukemia	Lungs	No	Primary disease

TABLE 1 (*Continued*)

MUCORMYCOSIS IN MAN

Case No.	Author	Age (yr.), Race and Sex	Geographic Location	Predisposing Factors	Organs Involved by Mucor	Cultural Verification	Cause of Death when Fatal
31	.....	38	Missouri	Hodgkin's disease	Lungs	No	Primary disease
32	.....	34	Missouri	Chronic lymphocytic leukemia	Lungs	No	Primary disease
33	.....	56	Missouri	Chronic myelogenous leukemia	Lungs	No	Primary disease
34	.....	Infant	Missouri	None	Brain, duodenum	No	Mucormycosis
35	.....	Infant	Missouri	None	Brain	No	Mucormycosis
36	.....	Adult	Missouri	None	Brain	No	Mucormycosis
37	McCall, Strobos, 1957 [*38*]	18,W,M	North Carolina	Diabetes; acidosis	Left eye, periorbital tissue, cavernous sinus (probable)	Rhizopus oryzae from purulent material from eye	Apparent recovery
38	Merriam, Tedeschi, 1957 [*46*]	57,W,M	Massachusetts	Diabetes; acidosis	Sphenoid sinus, orbit, pons, pituitary, meninges	No; attempts negative	Cerebral mucormycosis, diabetic acidosis
39	Mayfield, Condie, 1957 [*47*]	24,W,M	Not stated	Acute myelogenous leukemia; thrombocytopenia; cortisone, 6-mercaptopurine therapy	Lung	No	Myelogenous leukemia, pulmonary mucormycosis
40	Baker et al., 1957 [*17*]	43,W,M	North Carolina	Pulmonary tuberculosis; hepatitis; cortisone therapy	Stomach, liver, small and large intestine	No	Pulmonary tuberculosis, gastrointestinal mucormycosis with hemorrhage, hepatitis
41	.....	75,W,M	Mississippi	None identified	Cecum, ascending colon	No	Pneumonia, uremia, intestinal mucromycosis
42	Baker, 1957 [*27*]	62,W,M	Not stated	Cirrhosis of the liver	Brain, paranasal sinuses	No	Cerebral mucormycosis, subarachnoid hemorrhage
43	.....	Adult	Not stated	Diabetes with acidosis	Ethmoid sinus, eye (probable)	Rhizopus from biopsy of ethmoid sinus mucosa	Apparent recovery
44	Clark, 1957 [*48*]	59,W,M	Ohio	Diabetes; aplastic anemia; 6-mercaptopurine therapy	Duodenum, liver, cecum	No	Bronchopneumonia, mucormycosis
45	Jackson, Karnauchow, 1957 [*49*]	20 days, F	Canada	Congenital heart disease	Brain, meninges, ileum	No	Multiple congenital cardiac defects, cerebral and intestinal mucormycosis with perforation
46	Stefanini, Allegra, 1957 [*50*]	35,M	Massachusetts	Acute histiocytic leukemia; steroid, antibiotic therapy	Lung	No	Pulmonary mucormycosis, leukemia
47	Torack, 1957 [*51*]	64,W,M	New York	Multiple myeloma; steroid, antibiotic therapy	Heart, lungs, spleen, stomach, meninges	No	Multiple myeloma, uremia, suppurative meningitis and myocardial infarction due to mucor
48	.....	65,W,M	New York	Aplastic anemia; steroid, antibiotic therapy	Stomach	No	Pancytopenia
49	.....	50,W,M	New York	Plasma cell leukemia; Urethane,® steroid, antibiotic therapy	Lung	No	Pulmonary mucormycosis, uremia
50	Watson [*52*]	2 3/12,N,M	S. Africa	Kwashiorkor	Stomach	No	Gastric perforation secondary to mucormycosis
51	Bryan et al., 1958 [*53*]	2 10/12	Iowa	Diabetes with acidosis; pharyngitis	Brain, lungs, thymus, middle ear, intestine, aorta	Attempts unsuccessful	Disseminated mucormycosis, diabetic acidosis
52	Dwyer, Changus, 1958 [*54*]	46,W,F	Connecticut	Diabetes mellitus	Maxillary sinus, periorbital tissue, cavernous sinus, brain	No	Cerebral mucormycosis
53	Josefiak et al., 1958 [*55*]	27,W,M	North Carolina	Diabetes mellitus	Skin of leg	No	Recovery with healing of leg ulcer
54	Gatling, 1959 [*56*]	16 days,W,F	Virginia	Otitis media; antibiotic therapy	Stomach, liver, spleen, pancreas, colon, diaphragm, left lung	No	Mucormycosis
55	Fienberg, Risley, 1959 [*57*]	45,F	Massachusetts	Diabetes mellitus; appendiceal abscess	Aorta	No	Postoperative fever and shock, cause of death obscure
56	Case 1 (E. F.), 1959	32,W,F	Nova Scotia	Septic abortion; septicemia; cortical necrosis of kidneys, adrenals	Lungs, pharynx, larynx, esophagus, ileum	No	Disseminated mucormycosis, uremia, brain stem hemorrhages
57	Case 2 (R. S.), 1959	26,W,M	Massachusetts	Centrolobular hepatic necrosis; acute renal tubular nercosis; pancreatitis; uremia	Lungs, esophagus, stomach, ileum, colon	Rhizopus arrhizus from mesentery of small intestine	Hepatitis, uremia, disseminated mucormycosis

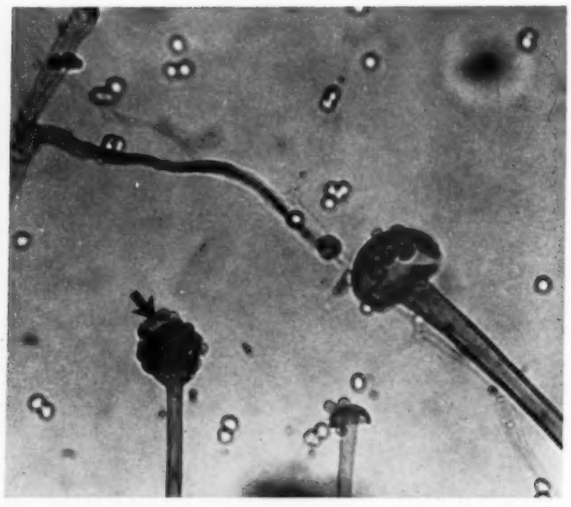


FIG. 8. Case II. R. arrhizus. Small spherical sporangiospores (arrow) liberated from spore case (sporangium). Sabouraud's medium, unstained, original magnification × 386.

phores) bearing sporangiospores. In mucor, no rhizoids are present at the point of attachment of the sporangiophores to the stolons. In rhizopus, the sporangiophores arise from the node of the stolons directly opposite the rhizoids.

The vegetative mycelium of the mucorales is composed of broad, branching, non-septate (coenocytic) hyphae measuring from 7 to 15 μ. in diameter.

The fungus is identified readily in hematoxylin and eosin or PAS-stained tissue sections by the characteristic morphology of the hyphae (Fig. 3) and by the propensity of the fungus to invade vascular channels. (Fig. 5.) Since sporulation requires aerobic conditions, spores are seen only occasionally in tissues, usually within the respiratory system. (Fig. 4.) The genus and species of the organism cannot be determined from tissue sections, but only after culture.

INFECTION IN MAN

Paltauf [*9*] in 1885 reported the first generally accepted valid case of mucormycosis in man. Fifty-seven acceptable cases of mucormycosis have been reported subsequently in the English literature and are summarized in Table I. The disease has been reported in males approximately twice as often as in females and in an age group ranging from sixteen days to seventy-five years.

Pathogenesis. The observation was made by Barthelet [*10*] in 1903, and has since been confirmed, that mucor is an invader of tissues previously altered by disease and is only rarely a primary pathogen.

In 1943 Gregory et al. [*11*] reported three cases of cerebral mucormycosis in patients with diabetic acidosis. It is evident from Table I that nearly half (42 per cent) of the cases occurred in diabetic patients, most of whom had been severely acidotic.

In non-diabetic patients the host of predisposing conditions incriminated includes leukemia, multiple myeloma, carcinoma, anemia, body burns, glomerulonephritis, acute tubular necrosis, septicemia, hepatitis, cirrhosis of the liver, congenital heart disease, diarrhea and uremia. The use of antibiotics, steroids, adrenal corticotropic hormone, folic acid antagonists and ionizing radiation likewise appears to favor the growth of the fungus [*12*]. Zimmerman [*13*] pointed out that in the past mucormycosis was closely associated with diabetes, but today it is encountered in pathologic states which predispose to aspergillus and candida infections. Although antibiotics are believed to modify the normal bacterial flora of the body, allowing ordinarily saprophytic fungi to become pathogenic, Kligman [*14*] in a review of this problem found no experimental data which would fully substantiate this view.

Why a usually saprophytic, non-pathogenic fungus becomes invasive is an interesting problem. Zimmerman [*13*] considered decreased host resistance, local lesions incident to the primary disease and disturbances brought about by therapy as factors which permit aggressive growth of the usually saprophytic fungi. It is evident that an alteration could take place in the fungus as a result of the primary disease, or a metabolic derangement or form of therapy which might render it pathogenic.

Clinical and Pathologic Features. In man, the mucorales have been responsible for lesions in many sites including the skin, nose, mouth, paranasal sinuses, stomach, small and large intestine, lungs, larynx, brain, eye, kidneys, liver, spleen, pancreas and bone marrow. Table II lists the frequency of organ involvement in the cases appearing in Table I. In 32 per cent, the brain alone was involved, in 28 per cent the lungs alone, in 7 per cent the gastrointestinal

tract was involved. In 28 per cent the disease assumed a disseminated form. Cerebral lesions have predominated in diabetic subjects. In non-diabetic, non-lymphoma patients, disseminated disease has occurred most frequently. In patients with lymphoma, pulmonary mucormycosis has been the most common. Our knowledge of the pathogenesis of this infection is too meager to explain the preponderance of cerebral involvement in the diabetic patient, of pulmonary involvement in the leukemic patient, and the more widespread infection in the non-diabetic, non-leukemic patient. In the cerebral form most patients present a syndrome consisting of diabetic acidosis, ophthalmoplegia and meningoencephalitis with lesions of mucormycosis in the brain and eye. In such patients the fungal spores probably gain entrance through the upper respiratory tract, proliferate into hyphae and produce a sinusitis and orbital cellulitis. Subsequently, the fungus invades and thromboses the ophthalmic and/or internal carotid arteries, extending to the brain either directly from the sinuses or via the blood vessels and cranial nerves [*15*]. The pathologic changes in the brain are of the nature of ischemic infarction secondary to mucorthrombosis of the vessels and meningoencephalitis secondary to direct invasion of the nervous tissue by the fungus.

In the pulmonary form, as discussed by Baker [*16*] in his excellent review, a primary pneumonitis occurs. The spores are inhaled, penetrate the bronchial wall and invade hilar tissues, parenchyma and blood vessels, causing mucorthrombosis and infarction of the pulmonary parenchyma. Changes in the lung indistinguishable from the primary form may result from hematogenous or lymphatic spread of organisms from remotely infected tissue. In the gastrointestinal form, fungi in the lumen penetrate the bowel wall and invade mesenteric vessels, producing ischemic infarction and making generalized dissemination possible [*17*].

In the disseminated form, which usually involves the brain, lungs and/or gastrointestinal tract, the portal of entry is thought to be either the respiratory or gastrointestinal mucosa.

Diagnosis. Clinical suspicion of cerebral mucormycosis should be aroused when a diabetic patient presents with orbital cellulitis, ophthalmoplegia and/or neurologic abnormalities suggestive of meningoencephalitis. The recovery of three diabetic patients in whom the diagnosis of cerebral mucormycosis was made during life, has been described; the first case was reported by Harris [*18*] in 1955.

TABLE II
FREQUENCY OF ORGAN INVOLVEMENT IN FIFTY-FOUR DIABETIC AND NON-DIABETIC PATIENTS WITH VISCERAL MUCORMYCOSIS*

Associated Disease	Site of Involvement			
	Brain	Lung	Gastro-intestinal Tract	Disseminated
Diabetes (21 patients)	14	3	0	4
No diabetes (33 patients)				
Leukemia or myeloma (13 patients)	0	11	0	2
No leukemia or myeloma (20 patients)	4	2	4	10

* Cases 11, 53 and 55, not involving viscera, are not included.

In pulmonary, gastrointestinal and disseminated mucormycosis, the diagnosis remains difficult because of its occurrence in the patient severely debilitated by another disease.

Laboratory Methods. The diagnosis of mucormycosis may be made by biopsy of accessible lesions, such as those occurring in the nasal or palatal mucosa or on the skin. The hyphae are identifiable readily in hematoxylin and eosin-stained sections. (Fig. 3.)

Biopsy material or scrapings from lesions should be cultured on Sabouraud's medium. The fungus produces an abundant, fluffy, white, filamentous colony within two to three days, which darkens in a few days. It is important that the laboratory be alerted to the possibility of mucormycosis, inasmuch as in one reported case a positive culture was discarded because the fungus was considered to be a laboratory contaminant.

Blood in clinically suspected cases should be cultured for fungi. Although positive cultures have not been reported in the literature, the frequency of hematogenous dissemination of the fungus suggests the possibility of diagnosis by this method.

A positive skin test to autogenous Rhizopus vaccine has been reported in one instance of mucormycosis [*8*]. The usefulness of such vaccines remains to be evaluated.

TABLE III

EXPERIMENTAL MUCORMYCOSIS

Author	Organism	Experimental Subjects	Route of Injection of Fungal Spores	Host Alteration	Sites of Significant *Mucor* Lesions	Comments
Bauer et al. [15].....	Rhizopus oryzae Rhizopus arrhizus Absidia ramosa Absidia corymbifera	Rabbits	Intranasal	Acute alloxan	Local (nose), brain, lung, kidney	
Elder, Baker [21].....	Rhizopus	Rabbits	Intrabronchial	Acute alloxan diabetes	Bronchi, lungs	Necrotizing lesions with vascular thrombosis and pneumonia
				Chronic alloxan diabetes	Lungs	Minimal pneumonia centered about spores; no hyphae produced; controls: lesions similar to those in chronic alloxan diabetes
Baker et al. [25].....	Rhizopus arrhizus	Rabbits	Intratracheal	Acute alloxan diabetes	Lungs	Similar lesions not found in chronic alloxan diabetic rabbits or mice
	Rhizopus oryzae	Mice, rats, guinea pigs	Intraperitoneal	Cortisone	Extraperitoneal tissues	
Schofield, Baker [58]...	Rhizopus arrhizus	Mice	Intraperitoneal	Chronic alloxan diabetes	Peritoneum	No extension beyond peritoneum in chronic alloxan diabetic animals
		Mice	Intracerebral	Chronic alloxan diabetes	Local reaction only, infarction in brain of one animal	
Bauer et al. [20].....	Rhizopus oryzae	Rabbits	Intranasal (spores injected 24 to 72 hours prior to alloxan)	Sustained hyperglycemia in non-diabetic subjects	Nose, lungs	
				Acute alloxan diabetes	Nose, lungs, brain	
Bauer, Sheldon [24]....	Rhizopus oryzae	Rabbits	Intranasal	Nitrogen mustard-induced leukopenia	Nose, lung, brain (rare)	Pronounced fungal growth only in first 2 to 4 days following injection of spores
Sheldon, Bauer [59]....	Rhizopus oryzae	Rabbits	Subcutaneous	Induction of acute alloxan diabetes following inoculation of spores	Local	Activation of fungus by acute alloxan diabetic state

MUCORMYCOSIS IN THE EXPERIMENTAL ANIMAL

Lichtheim [*19*], in 1884, in the first experimental approach to this disease, gave intravenous injections of spores of Mucor corymbifer and M. rhizopodiformis to rabbits, producing widespread lesions and death. Spontaneously occurring lesions have been reported in birds, dogs, swine, horses and cows [*11*].

Studies of experimental mucormycosis are summarized in Table III. Bauer et al. [*15*] gave intranasal injections of spores of rhizopus and absidia to rabbits with acute alloxan diabetes and produced significant lesions in the nose, brain, lung and kidney. Elder and Baker [*21*] demonstrated that in the rabbit acute alloxan diabetes was necessary for the invasiveness of rhizopus. Hyperglycemia was not considered an important predisposing factor, since in their study rabbits in which chronic alloxan diabetes had been induced, with hyperglycemia but without ketosis, resisted infection as successfully as the normal control subjects. They postulated that the increased susceptibility of rabbits with acute alloxan diabetes to rhizopus is due to ketosis with associated loss of phagocytic ability of macrophages and giant cells, and changes in leukocyte metabolism.

Bauer et al. [*20*], after giving an injection of R. oryzae spores into non-diabetic rabbits in which infusion hyperglycemia had been maintained, found significant nasal and pulmonary lesions, indicating that hyperglycemia alone allows rhizopus to become invasive. Lesions in the nose, lung and brain were produced in rabbits given an injection of rhizopus spores twenty-four hours before the alloxan, and in the nose and lungs of animals given an injection seventy-two hours prior to alloxan, demonstrating the persistence of potentially pathogenic spores in the normal animal. These authors suggested that the diminished fungicidal power of the neutrophil is the common denominator in both hyperglycemia without diabetes and in acute alloxan diabetes and that this may be a factor in the diminished resistance of the host.

The role of the inflammatory response in the increased susceptibility to infection in rabbits made acutely diabetic by alloxan has been studied by Cruickshank [*22*], who found a failure of the inflammatory response to coagulase-positive micrococci. However, Schauble and Baker [*23*] observed an excellent inflammatory response to both coagulase-positive micrococci and rhizopus in the same experimental model and concluded that a quantitative defect in neutrophils in alloxan diabetes does not contribute to the increased susceptibility to rhizopus infection.

The importance of the neutrophil in experimental mucormycosis was studied by Bauer and Sheldon [*24*] in rabbits in which leukopenia had been induced by means of nitrogen mustard. They found that leukopenia decreased host resistance only during the early stage of the infection. In the later stages of the disease the lesions tended to regress spontaneously, indicating unimpaired host resistance.

Baker et al. [*25*] produced evidence that mice, rats and guinea pigs treated with cortisone were more susceptible to mucor infection than non-treated control animals.

Josefiak and Foushee [*26*] gave an injection of R. oryzae into a Selye pneumoderm in healthy rats and found that R. oryzae produced a granulomatous reaction in six days. In four to six weeks healing had begun and there was no evidence of spread of the infection beyond the pneumoderm.

One of us has recently observed the spontaneous occurrence of mucormycosis in rabbits who became severely leukopenic following lethal doses of radiation to the whole body.

TREATMENT

Three patients in whom the diagnosis of mucormycosis was made during life recovered. Harris [*18*] reported the first case in which recovery occurred, that of a fourteen year old Negro girl with diabetic acidosis, and mucormycosis involving the palate, left eye and cranial nerves. R. arrhizus was cultured from a palatal biopsy specimen. The patient gave a positive skin test to a vaccine prepared from this agent. Treatment included desensitization with the vaccine, rigid control of the diabetes and the administration of potassium iodide. Baker [*27*] described an adult with diabetic acidosis and an acute ethmoid sinusitis from whom rhizopus was cultured. Recovery resulted with regulation of the diabetes. McCall and Strobos [*28*] reported a case of mucormycosis in an eighteen year old diabetic boy with pansinusitis, orbital cellulitis and probable cavernous sinus thrombosis due to R. oryzae. Treatment consisted of regulation of the diabetes, and therapy with

Mycostatin,® Parenzyme,® potassium iodide, anticoagulants and antibiotics. Chick et al. [*29,30*] found that amphotericin B has an inhibitory effect on the course of rhizopus infection in the rabbit.

On the basis of current limited clinical and experimental data, the following guides to treatment of mucormycosis may be emphasized: (1) regulation of diabetes or other underlying disease, (2) isolation of the fungus and determination of the sensitivity of the fungus to agents of potential value, (3) re-evaluation of the continued use of antibiotic and steroid therapy and (4) consideration of desensitization with autogenous vaccines, and of the administration of amphotericin B and of iodides.

SUMMARY

1. Two cases of mucormycosis, a usually fatal infection reported with increasing frequency, are presented. In one, a young woman with postabortal necrosis of the spleen, kidneys and adrenals, and severe uremia, mucormycosis was thought to contribute to the fatal outcome. The second case was a young man with hepatic disease, possibly toxic in origin, and ischemic tubular necrosis. In both cases mucormycosis was disseminated, involving the respiratory and gastrointestinal tracts.

2. Rhizopus arrhizus was identified in the second case. This is the sixth case of visceral mucormycosis in which the organism has been identified by culture.

3. Clinical and pathologic features of fifty-five cases of mucormycosis reported in the English literature are summarized. In twenty-one of these cases diabetes was present and in thirteen, leukemia or lymphoma. The role of these conditions, as well as that of cortisone and antibiotics in the pathogenesis of mucormycosis, is discussed.

4. The most frequently involved sites have been the brain and meninges, usually in diabetic patients. Pulmonary and disseminated forms have predominated in non-diabetic subjects.

5. The diagnosis of mucormycosis should be suspected in the diabetic patient who presents a triad of orbital cellulitis, ophthalmoplegia and meningoencephalitis. Some of these patients may have ulceration of the nasal mucosa and here the diagnosis may be established by histologic examination and culture of biopsy material or scrapings. The diagnosis in the pulmonary and gastrointestinal forms of infection remains difficult.

6. The experimental data are summarized. Alterations resulting from acute alloxan diabetes, acidosis in the form of ketosis, leukopenia, hyperglycemia and cortisone administration have been found to decrease host resistance to mucormycosis.

7. Recovery has been reported in three patients with cerebral mucormycosis. The treatment recommended includes control of the underlying disease, use of vaccines, amphotericin B and iodides.

Acknowledgment: We wish to express our gratitude to Mr. James B. Gray, Mycologist, State of Massachusetts Diagnostic Laboratory, Boston, Massachusetts for preparation of the original cultures in Case II and to Dr. C. W. Hesseltine of the U. S. Agricultural Research Service, Peoria, Illinois, for taxonomic classification of the organism. Dr. Hesseltine has entered this culture as number A-8077 in his collection.

REFERENCES

1. McKay, D. G., Merrill, S. J., Weiner, A. E., Hertig, A. T. and Reid, D. E. The pathological anatomy of eclampsia, bilateral renal cortical necrosis, pituitary necrosis and relation to the Shwartzman phenomenon. *Am. J. Obst. & Gynec.*, 66: 507, 1953.
2. Sheldon, W. H. and Hertig, A. T. Bilateral cortical necrosis of the kidney. *Arch. Path.*, 34: 866, 1942.
3. Hesseltine, C. W. Personal communication.
4. Gray, J. B. Personal communication.
5. Hesseltine, C. W. A survey of the Mucorales. *Tr. New York Acad. Sc.*, 14: 210, 1952.
6. Garrett, S. D. Ecological groups of soil fungi. *New Phytologist*, 50: 149, 1951.
7. Morwood, R. B. Peanut diseases. *Queensland Agriculture J.*, 79: 267, 1954.
8. Miller, J. H., Gidden, J. E. and Foster, A. A. A survey of the fungi of forest and cultivated soils of Georgia. *Mycologia*, 49: 779, 1957.
9. Paltauf, A. Mycosis mucorina. *Arch. path. Anat.*, 102: 543, 1885.
10. Barthelet, C. J. Les mucorines pathogènes et les mucormycosis. *Arch. de Parasitol. Par.*, 7: 5, 1903.
11. Gregory, J. E., Golden, A. and Haymaker, W. Mucormycosis of the central nervous system. *Bull. Johns Hopkins Hosp.*, 73: 405, 1943.

12. Craig, J. M. and Farber, S. The development of visceral mycosis during therapy for acute leukemia. *Am. J. Path.*, 29: 601, 1953.
13. Zimmerman, L. E. Fatal fungus infections complicating other diseases. *Am. J. Clin. Path.*, 25: 46, 1955.
14. Kligman, A. M. Are fungus infections increasing as a result of antibiotic therapy? *J. A. M. A.*, 149: 979, 1952.
15. Bauer, H., Flanagan, F. J. and Sheldon, W. H. Experimental cerebral mucormycoses in rabbits with alloxan diabetes. *Yale J. Biol. & Med.*, 28: 29, 1955.
16. Baker, R. D. Pulmonary mucormycosis. *Am. J. Path.*, 32: 287, 1956.
17. Baker, R. D., Bassert, D. E. and Ferrington, E. Mucormycosis of the digestive tract. *Arch. Path.*, 63: 176, 1957.
18. Harris, J. S. Mucormycosis. *Pediatrics*, 16: 857, 1955.
19. Lichtheim, L. Über pathogene Mucorineen und die durch sie erzeugten Mykosen des Kaninchens. *Ztschr. klin. Med.*, 7: 140, 1884.
20. Bauer, H., Flanagan, J. F. and Sheldon, W. H. Metabolic alterations in mucormycosis infection. *Yale J. Biol. & Med.*, 29: 23, 1956.
21. Elder, T. D. and Baker, R. D. Pulmonary mucormycosis in rabbits with alloxan diabetes. *Arch. Path.*, 61: 159, 1956.
22. Cruickshank, A. S. Resistance to infection in the alloxan-diabetic rabbit. *J. Path. & Bact.*, 67: 323, 1954.
23. Schauble, M. K. and Baker, R. D. The inflammatory response n acute alloxan diabetes. *Arch. Path.*, 64: 563, 1957.
24. Bauer, H. and Sheldon, W. H. Leucopenia and experimental mucormycosis (Rhizopus oryzae infection). *Am. J. Path.*, 33: 617, 1957.
25. Baker, R. D., Schofield, R. A., Elder, T. D. and Spoto, A. P. Alloxan diabetes and cortisone as modifying factors in experimental mucormycosis (Rhizopus infection). *Fed. Proc.*, 15: 506, 1956.
26. Josefiak, E. J. and Foushee, J. H. S. Experimental mucormycosis in the healthy rat. *Science*, 127: 1442, 1958.
27. Baker, R. D. Mucormycosis—a new disease? *J. A. M. A.*, 163: 805, 1957.
28. McCall, W. and Strobos, R. J. Survival of a patient with central nervous system mucormycosis. *Neurology*, 7: 290, 1957.
29. Chick, E. W., Evans, J. and Baker, R. D. Treatment of experimental mucormycosis (Rhizopus oryzae infection) in rabbits with amphotericin B. *Antibiotics & Chemother.*, 8: 394, 1958.
30. Chick, E. W., Evans, J. and Baker, R. D. The inhibitory effect of amphotericin B on localized Rhizopus oryzae infection (mucormycosis) utilizing the pneumoderma pouch of the rat. *Antibiotics & Chemother.*, 8: 506, 1958.
31. LeCompte, P. M. and Meissner, W. H. Mucormycosis of the central nervous system associated with hemochromatosis. *Am. J. Path.*, 23: 673, 1947.
32. Moore, M., Anderson, W. A. D. and Everett, H. H. Mucormycosis of the large bowel. *Am. J. Path.*, 25: 559, 1949.
33. Lloyd, J. B., Sexton, L. I. and Hertig, A. T. Pulmonary mucormycosis complicating pregnancy. *Am. J. Obst. & Gynec.*, 58: 548, 1949.
34. Wolf, A. and Cowen, D. Mucormycosis of the central nervous system. *J. Neuropath. & Exper. Neurol.*, 8: 107, 1949.
35. Stratemeier, W. P. Mucormycosis of the central nervous system: Report of a case. *Arch. Neurol. & Psychiat.*, 63: 179, 1950.
36. Murphy, J. D. and Barnstein, S. Mucormycosis of the lung. *Ann. Int. Med.*, 33: 442, 1950.
37. Wadsworth, J. A. C. Ocular mucormycosis. *Am. J. Ophth.*, 34: 405, 1951.
38. Martin, F. P., Lukeman, J. M., Ranson, R. F. and Geppert, L. J. Mucormycosis of the central nervous system associated with thrombosis of the internal carotid artery. *J. Pediat.*, 44: 437, 1954.
39. Kurrein, F. Cerebral mucormycosis. *J. Clin. Path.*, 7: 141, 1954.
40. Bauer, H., Ajello, L., Adams, E. and Hernandez, D. V. Cerebral mucormycosis; pathogenesis of the disease (description of Rhizopus oryzae). *Am. J. Med.*, 18: 822, 1955.
41. Gunson, H. H. and Bowden, D. H. Cerebral mucormycosis; report of a case. *Arch. Path.*, 60: 440, 1955.
42. Foushee, S. and Beck, W. C. Mucormycosis of the central nervous system; case report. *North Carolina M. J.*, 17: 26, 1956.
43. Baker, R. D. and Severance, A. O. Mucormycosis with report of acute mycotic pneumonia. *Am. J. Path.*, 24: 716, 1948.
44. Clinical pathological conference. *M. J. Australia*, 2: 30, 1956.
45. Keye, J. D. and Magee, W. E. Fungal diseases in a general hospita.; study of 88 patients. *Am. J. Clin. Path.*, 26: 1235, 1956.
46. Merriam, J. C. and Tedeschi, C. G. Cerebral mucormycosis. *Neurology*, 7: 510, 1957.
47. Mayfield, G. R. and Condie, F. Paradoxical mucorthrombosis in thrombocytopenia purpura. *Arch. Path.*, 63: 260, 1957.
48. Clark, R. M. A case of mucormycosis of the duodenum, liver and cecum. *Gastroenterology*, 33: 985, 1957.
49. Jackson, J. R. and Karnauchow, P. N. Mucormycosis of the central nervous system. *Canad. M. A. J.*, 76: 130, 1957.
50. Stefanini, M. and Allegra, S. Pulmonary mucormycosis in acute histiocytic leukemia. *New England J. Med.*, 256: 1026, 1957.
51. Torack, R. M. Fungus infections associated with antibiotic and steroid therapy. *Am. J. Med.*, 22: 872, 1957.
52. Watson, K. C. Gastric perforation due to the fungus Mucor in a child with Kwashiorkor. *South African Med. J.*, 31: 99, 1957.
53. Bryan, G. T., Read, C. H. and Zimmerman, G. R. Disseminated mucormycosis in a child with diabetes mellitus. *J. Iowa M. Soc.*, 48: 193, 1958.

54. Dwyer, G. K. and Changus, G. W. Rhinomucormycosis resulting in fatal cerebral mucormycosis. *Arch. Otolaryng.*, 67: 619, 1958.
55. Josefiak, E. J., Foushee, J. H. S. and Smith, L. C. Cutaneous mucormycosis. *Am. J. Clin. Path.*, 30: 547, 1958.
56. Gatling, R. R. Gastric mucormycosis in a newborn infant. *Arch. Path.*, 67: 249, 1959.
57. Fienberg, R. and Risley, T. S. Mucormycotic infection of arteriosclerotic thrombus of the abdominal aorta. *New England J. Med.*, 260: 626, 1959.
58. Schofield, R. A. and Baker, R. D. Experimental mucormycosis (Rhizopus infection) in mice. *Arch. Path.*, 61: 407, 1956.
59. Sheldon, W. H. and Bauer, H. Activation of quiescent mucormycotic granuloma in rabbits by induction of acute alloxan diabetes. *J. Exper. Med.*, 108: 171, 1958.

Case Reports

"Kwashiorkor-Like" Syndrome, Associated with Burning Feet Syndrome, in an Adult Male*

CHARLES W. SILVERBLATT, M.D. *and* HARVEY E. BROWN, JR., M.D.
Coral Gables, Florida

To our knowledge, severe nutritional deficiency producing a kwashiorkor-like syndrome has not been reported in the adult male in the United States. Mellinkoff [*1*] reported one case of temporary redness of the hair in a patient with ulcerative colitis, Krosnick [*2*] described two cases of gonadal dysfunction in two females with severe nutritional deficits, and Perloff et al. [*3*] reported five cases of functional hypopituitarism due to malnutrition, but none of these cases were characterized by a kwashiorkor-like state.

The following case represents what we believe is the first report of such a syndrome without a demonstrable complicating disease in an adult male native-born inhabitant of the United States.

CASE REPORT

J. A., a forty-five year old Negro man, born in a rural community in North Florida, was admitted to the V. A. Hospital for the fourth time on January 26, 1958, with a chief complaint of burning pain, particularly nocturnal, of both feet for six months. He stated that he had been well until approximately one and a half years previously, when he began to lose weight and severe diarrhea with general lassitude, weakness, anemia, impotence, swelling of the ankles and "peeling of the skin" had developed progressively. The diarrhea was described as persistent, unrelated to time of day, porridge-like in consistency and appearance, and containing occasionally undigested food particles. The latter was not observed at any time during his prolonged hospitalization. The patient claimed to have lost 50 pounds during the previous six months. During this time he had also lost all the hair on his body and most of the hair on his head, which had been black. Four months prior to this admission the patient had entered another hospital where a diagnosis of idiopathic steatorrhea with secondary anemia and congestive heart failure was made. Information obtained from that hospital revealed that both the total and free fat in the stools was increased, and meat fibers were present. The serum albumin was 2 gm. per cent and globulin 2.5 gm. per cent. An oral cobalt-labeled vitamin B_{12} study revealed 6.2 per cent absorption. Glucose tolerance was described as normal. Bromsulfalein retention was 25 per cent on admission and 5 per cent at the time of discharge. The patient was treated with a low fat, high protein, high carbohydrate diet supplemented with vitamins and minerals. ACTH and prednisone were given but use of the drugs was subsequently discontinued because of the development of mental symptoms, severe edema and pulmonary congestion. Further interrogation revealed that the patient felt somewhat improved while receiving steroids, but that he did not choose to take them again because they "made me feel crazy."

It was during this hospitalization that desquamation of the skin was first noticed; this occurred prior to the administration of steroids. The desquamation was complete, so that over an interval of three months the patient gradually shed his entire skin surface. Following discharge his symptoms became worse, and when painful feet developed he sought admission to the hospital.

Past medical history revealed that at the age of seventeen he had had an attack of articular inflammation compatible with acute rheumatic fever. He admitted to having had gonorrhea in 1938.

The patient had been previously studied in this hospital in 1946 with complaints of epigastric distress, weight loss and vomiting. A diagnosis of duodenal ulcer was made, and since the patient failed to respond to a medical regimen a subtotal gastric resection was performed on September 30, 1946, with an

* From the General Medical Section, V. A. Hospital, and the University of Miami School of Medicine, Coral Gables, Florida.

FIG. 1. At the time of admission, skin is pink except for pigmentary changes of both lower extremities, as well as absent body hair.

anterior Polya type of repair. Three months later he returned for right inguinal herniorrhaphy and appendectomy. He was readmitted in February 1947 with symptoms suggestive of a postgastrectomy dumping syndrome. X-ray study of the upper gastrointestinal tract revealed the partial gastrectomy, with normal function except for a rapid emptying time. The patient was discharged on May 10, 1947, on a dietary program. He was not seen again until May 24, 1955, when he was treated at this hospital for fracture of the right lateral malleolus. At that time it was noted that the patient was not having any complaints referable to his gastrointestinal system and had been consuming a regular diet for several years.

There was a strong history of alcoholic excess up to 1955, at which time he stated he stopped alcoholic beverages of all types. His occupation prior to his present illness was that of presser in a cleaning establishment, but he denied using cleaners or detergents containing carbon tetrachloride.

Family history disclosed that his mother, father and several siblings were living and well. He is married and the father of two children, apparently healthy.

On physical examination, the temperature was 97.6°F., the pulse 90 per minute and the blood pressure in the supine position 110/80 mm. Hg, in the upright position 90/70 mm. Hg; his weight was 108 pounds (average normal weight 160 pounds).

The patient was a well developed, poorly nourished Negro man, chronically ill but alert and cooperative. He was extremely weak, requiring assistance to maintain the upright position. The mucous membranes of the mouth were pale and the tongue was slick but not red. There was some purulent pharyngeal secretion but no evidence of cheilosis, jaundice, conjunctivitis or keratitis. The neck veins were not distended and the thyroid was not palpable. On his head and upper lip there was only a fine lanugo-like hair, which had a definite reddish tinge. The eyebrows and eyelashes were entirely absent. The skin of the face was absolutely smooth. The lungs were clear to percussion and auscultation. The heart was not enlarged. There were no thrills. A grade 3 systolic murmur was heard at the apex and transmitted to the axilla. A grade 3 presystolic murmur was also noticed at the apex in the left lateral recumbent position. No opening snap was heard. The rhythm was regular. The pulmonic second sound was accentuated and closely split. The abdomen was scaphoid in appearance. The liver edge was palpable 1 fingerbreadth below the right costal margin and non-tender. No masses were noted. There was an upper right rectus scar and a right inguinal scar, both well healed. Rectal examination revealed the prostate to be smaller than normal. There were no masses. A soft, yellowish stool was present, which was negative for blood. Both testicles were small and soft. The penis appeared normal. There was no pubic hair. There was 2-plus brawny edema of the lower extremities, with pigmentary and stasis-like changes of both ankles. (Fig. 1.) The peripheral pulses were normal. There was a dime-sized ulcer with a purulent base over the right lateral malleolus. The nails were grossly misshapen with transverse ridges at the base, absent moons and linear striations. The soles of the feet were very tender. There was no axillary hair. The skin was dry and no sweating was noted. There was a pinkish tinge to the entire body. Generalized desquamation was present and there was marked loss of turgor. There was no lymphadenopathy. Neurologic examination revealed no pathologic findings except that the deep tendon reflexes were generally decreased and the ankle jerks could not be elicited. There was no impairment of sensation. Vibratory sense appeared to be intact. Muscular wasting and weakness of the extremities was evident.

On admission the white blood count was 10,200 per cu. mm. with a normal differential. The hemoglobin was 10.5 gm. per cent (12 gm. per cent at the time of discharge). The hematocrit was 37.5 per cent on admission (37 per cent at discharge). The blood indices were normal. Urinalysis revealed an acid reaction; specific gravity 1.015; albumin negative; sugar negative; and microscopic examination showed an occasional white blood cell. Bromsulfalein retention was 18 per cent at the end of thirty minutes. At the time of discharge a repeat of this procedure revealed 2.2 per cent retention after thirty minutes.

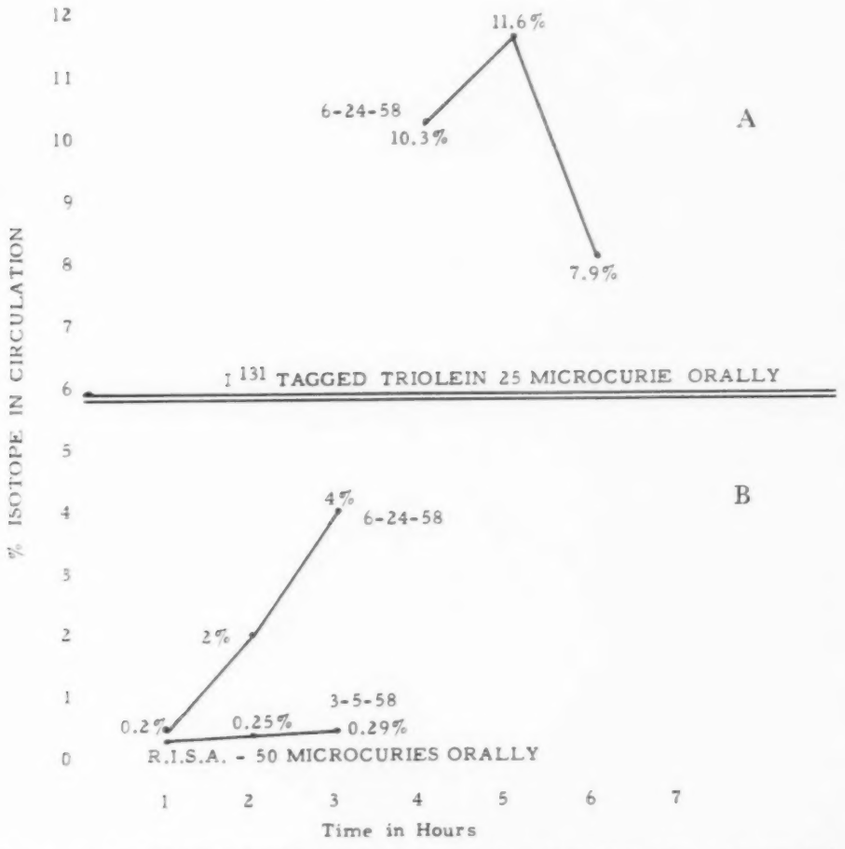


FIG. 2. A, normal labeled-triolein uptake at the time of discharge from the hospital (August 4, 1958). B, marked improvement of intestinal absorption of radioiodinated serum albumin after oral administration of 50 μc. at the time of discharge, as compared to absorption of the same dose at the time of admission.

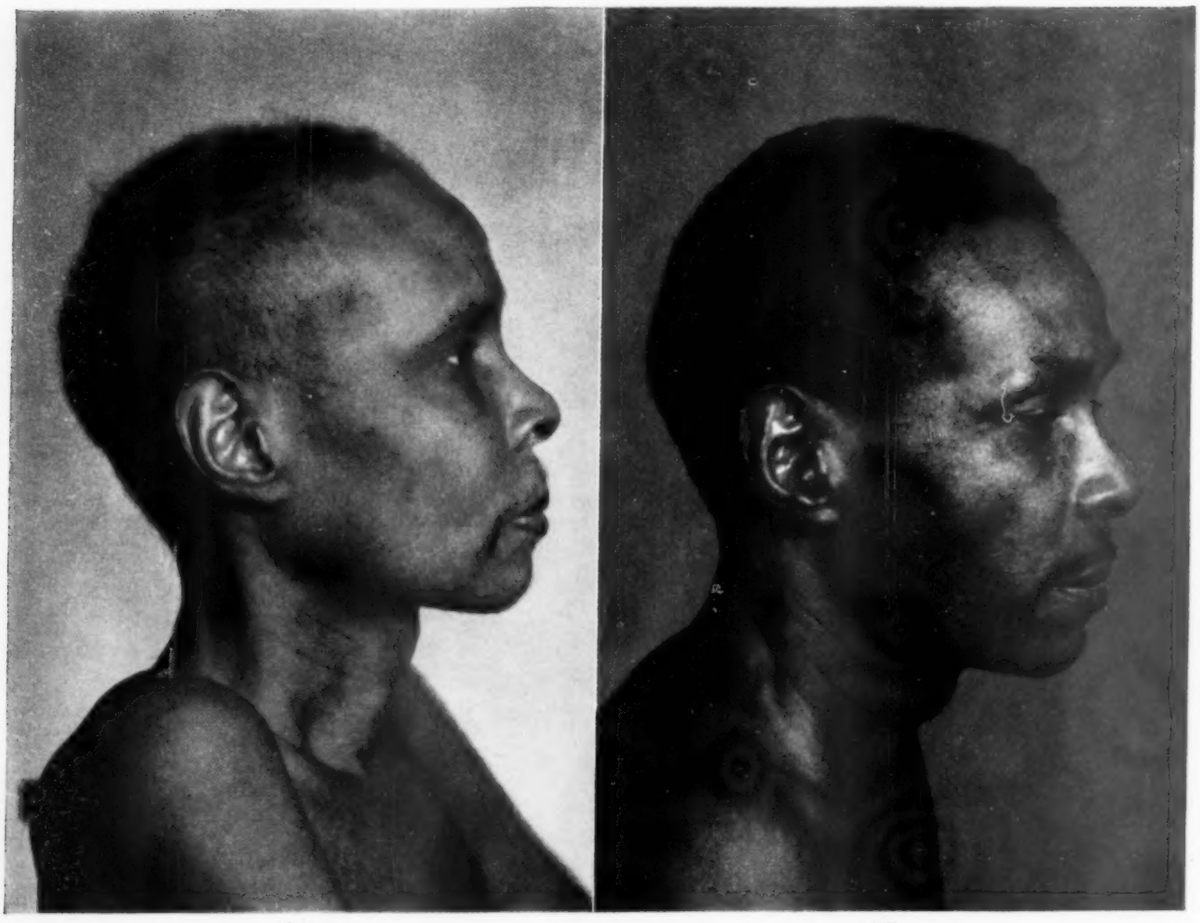
The serum cholesterol was 100 mg. per cent, cholesterol esters 80 mg. per cent; on discharge the serum total cholesterol was 235 mg. per cent, cholesterol esters 144 mg. per cent. The cephalin flocculation test was 1 plus. The prothrombin time was 15 seconds with a control of 14 seconds. The serum sodium was 128, potassium 3.76, chloride 105, and bicarbonate 24 mEq. per liter. The serum calcium was 9.4 mg. per cent, serum inorganic phosphorus was 3.82 mg. per cent. The blood urea nitrogen was 10 mg. per cent. The plasma 17-hydroxycorticosteroid was 2 gamma per 100 ml. On admission the 17-ketosteroid excretion was 5.6 mg. per twenty-four hours with a urine volume of 1,110 cc., at the time of discharge the 17-ketosteroid excretion was 19 mg. per twenty-four hours with a urine volume of 1,260 cc. Radioactive iodine uptake by the thyroid was 9 per cent of the administered dose after twenty-four hours; on followup examination May 1, 1959 the radioactive uptake was 8 per cent after twenty-four hours. The circulating eosinophils were 53 per cu. mm. and after the administration of 25 mg. ACTH intravenously over a four-hour period were found to be 50 per cu. mm. At the time of follow-up examination (May 1, 1959) a similar determination revealed circulating eosinophils to be 147 per cu. mm. and 50 per cu. mm. after stimulation with ACTH.

An oral glucose tolerance test revealed a fasting blood sugar of 87 mg. per 100 ml.; a rise to 158 after thirty minutes, 190 at one hour, 195 at two hours, and 102 at three hours. The urine showed 1-plus sugar reaction in the one-hour and two-hour specimens. At follow-up examination the fasting blood sugar was

TABLE 1
SERUM PROTEINS DURING HOSPITALIZATION

Date (1958)	Albumin (gm. %)	Globulin (gm. %)	Total Serum Protein (gm. %)
2/12	1.8	2.7	4.5
2/28	1.7	3.1	4.8
3/28*	2.6	3.15	5.75
4/10	2.8	3.65	6.45
4/29	2.85	4.35	7.2
6/25	3.85	3.63	7.48

* Plasma administration started 500 cc. daily administered intravenously for twenty-one days.



3A 3B

FIG. 3. A, at the time of admission the hair is finely textured and reddish brown. B, at the time of discharge the hair is of normal texture and natural black.

85 mg. per 100 ml.; 30 minutes, 125; one hour, 145, two hours, 105 and three hours, 88. The urine was negative for sugar in all specimens.

Multiple stool examinations were negative for ova, parasites, occult blood and enteric pathogens. Bone marrow study revealed only erythroid hyperplasia. A chest roentgenogram was reported as within normal limits and barium study of the gastrointestinal tract was reported as showing a normally functioning gastroenterostomy stoma with barium entering both stomal loops. Serial films showed normal gastric emptying as well as normal small bowel pattern and motility. The colon was described as within normal limits. Sigmoidoscopy revealed only a pale mucosa with no ulcerations or bleeding points. A bone survey was within normal limits. The electrocardiogram showed non-specific T wave changes and borderline first degree A-V block with a PR interval measuring 0.21 to 0.22 seconds. Cardiac fluoroscopy revealed no chamber enlargements.

An oral radioiodinated serum albumin test (50 μc. orally in a fasting state) showed only 0.29 per cent absorption after three hours and at the time of discharge a repeat of this examination revealed 4 per cent absorption at the end of three hours. (Fig. 2B.) The serum total proteins on admission were 4.5 gm. per 100 ml., albumin 1.8 gm. per 100 ml., globulin 2.7 gm. per 100 ml. Other determinations of serum proteins are represented in Table 1.

The patient was given a low fat, high protein, high carbohydrate diet with vitamin supplements, and he demonstrated at all times an excellent appetite, frequently requesting additional food. He continued to have four to six stools daily, all appearing as a "light yellow to tan porridge." No undigested food particles were observed grossly or microscopically at any time, nor were the stools particularly foul-smelling or bulky. They were not restricted to any particular time of day. On bedrest the dependent edema of the lower extremities disappeared. The ulcer on the right ankle healed satisfactorily, but the skin continued to desquamate. He had no specific complaints, except severe weakness and the aggravating symptom of burning of the feet, particularly at night. No penile erections were reported during the patient's stay in the hospital. Hair growth on the head and upper lip continued to be of the soft lanugo type, and at all times manifested a definite reddish tinge. (Fig. 3A.) He was afebrile and

spent most of the time lying in bed because of severe weakness and mild postural hypotension.

One month following admission, during an epidemic of influenza, the patient had an acute infection of the upper respiratory tract with temperature elevation to 101°F. (orally) for two days. Treatment was supportive. He tolerated the illness quite well and actually had one of the most benign courses of any of the patients on the ward in whom this infection developed.

The general consensus of the staff was that the patient had an idiopathic malabsorption defect or abnormal gastrointestinal fistula. A consultant in dermatology first suggested the possibility of kwashiorkor.

With the possibility in mind of a blind loop syndrome, the patient was treated for a period of two weeks with Achromycin® (1 gm. administered daily in divided doses) but this was without any appreciable benefit. There was a gradual gain in weight, so that two months after admission he weighed 114 pounds.

A liver biopsy using a Vim-Silverman needle was performed on April 9, 1958 and the pathologic report was "marked fatty infiltration, hemosiderosis, partial fibrosis and focal hepatic cell degeneration." These changes were considered consistent with advanced nutritional deficiency. In view of a report in *Lancet* on March 8, 1958 [*4*] it was decided to explore this patient for the possibility of a gastrocolic fistula or a blind loop syndrome. In preparation for laparotomy an attempt was made to build up the plasma proteins as much as possible. To accomplish this the patient was given 500 cc. of plasma daily for twenty-one days. During this interval the patient showed the first signs of real improvement, consisting of a definite decrease in weakness, lassitude and skin changes. In spite of this the proposed surgery was accomplished on April 18, 1958. At operation a thorough search for any abnormal fistula or blind loop was made but no abnormality was noticed. The pancreas was palpated and felt to be normal, the liver was described as questionably granular but of normal consistency and not grossly enlarged. Postoperatively a bilateral parotitis developed which responded rapidly to radiation therapy. Otherwise the postoperative course was uneventful. The patient continued to eat well. The diarrhea diminished, the skin improved remarkably, and he began to display some normal appearing black hair on the upper lip and in both axillae. Weight gain was progressively improved, so that by May 16, 1958 he weighed 138 pounds and by June 9 his weight was 145 pounds. By this time the skin was completely clear, the patient was freely ambulatory, postural hypotension was no longer demonstrable, and there was no edema. He was given a diet containing 4,000 to 4,500 calories per day. However, ambulatory patients at this hospital have access to a canteen and it was noticed by the attendants that the patient was a frequent visitor and apparently consumed many more calories than his daily dietary allowance. He continued, however,

FIG. 4. At the time of discharge skin is normal brown and body hair has returned.

to complain of burning pain in both lower extremities, particularly his feet, although he admitted this was much less severe than at the time of admission. In view of the apparent marked improvement in his well-being, he was discharged on August 4, 1958.

He was next seen in the outpatient clinic on August 27, 1958. At that time he felt and looked quite healthy. (Figs. 3B and 4.) He was having only one bowel movement per day and that appeared normal to the patient. He had had intercourse with ejaculation on two occasions. His weight was 149 pounds. His appetite was excellent but he still experienced fatigue with mild exertion. The burning of the feet was markedly improved. His blood pressure was 110/80 mm. Hg supine and 115/90 mm. Hg upright. Multiple vitamins were prescribed and he was advised to lead an essentially normal life. He was next seen on October 30, 1958, at which time there was no overt manifestation of previous illness. He had normal hair distribution, typical in color (black) and consistency, and he shaved daily. He was having frequent erections. Bowel movements continued to be normal. There was still mild residual weakness with exertion. The previously described heart murmurs were no longer discernible and an electrocardiogram was interpreted as within normal limits.

COMMENTS

There seems to be some confusion in the literature as to just what the word "kwashiorkor" means. It is usually considered to represent "red boy," referring either to the hair or to the skin. However, from the medical department of the University of Kampala comes the statement that the word kwashiorkor means literally "weaned boy" [5] and perhaps was suggested by the fact that the syndrome of kwashiorkor was most likely to develop following weaning of the infant from the breast.

The syndrome of kwashiorkor is usually seen in tropical countries or in areas of severe famine. If this syndrome represents only a severe protein deficiency it should be fairly frequent in the United States, and perhaps it has been overlooked in the past. It is usually characterized by marked fatty infiltration of the liver and fibrosis and atrophy of the pancreas, together with edema and diarrhea. Accompanying the severe nutritional deficiency is a generalized desquamative dermatitis and reddening of the hair. If it is not allowed to progress too long, the syndrome is easily interrupted by proper administration of protein.

Krikler and Schrire [4] reported a case of kwashiorkor in an adult due to an intestinal blind loop. The case was in almost every respect identical with the one in this report with the one important exception, *viz.*, we were unable to find any anatomic cause for the diarrhea and the resulting nutritional deficiency. Their patient had a marked decline in the serum albumin as well as changes suggestive of hormonal deficiency, although they did not report cortical steroid or ketosteroid determinations. The response of their patient following surgical revision was remarkable, just as the response of the patient in our report was remarkable following the administration of large amounts of plasma protein.

Melinkoff [1], reporting a case of severe ulcerative colitis, described a white man whose hair turned red during the height of the nutritional failure that accompanied the disease. With the improvement of his condition the color of the hair returned to normal. However, his patient apparently had not reached the severe nutritional decline demonstrated in both Krikler's [4] case and ours, and hepatomegaly and dermatitis were not present. Endocrine studies were not obtained and the serum albumin did not reach the low level seen in our case.

Leitner and co-workers [6] reported two cases of a protein malnutrition syndrome similar to the one under discussion, except that both cases followed resection of the head of the pancreas and cholecystogastrostomy. A rather severe malabsorption state developed with marked protein deficiency. One of their patients died; the second, a fifty-nine year old woman, survived following dietary regulation with a very high protein intake. The skin changes described were similar to those of our patient, but red hair was not observed. Glossitis and cheilosis were present to a marked degree in both patients, and these were conspicuous by their absence in our case.

It has been well documented that vitamin requirements are directly proportional to effective caloric intake. Consequently, vitamin deficiency states are unlikely to occur in patients with any type of seriously impaired caloric intake. Starvation states in Europe during World War II and in the Minnesota experiment were characterized by a surprising lack of vitamin deficiency syndromes [7]. However, the patient's chief complaint, namely "nocturnal burning of the feet," is usually considered to be the result of vitamin B complex deficiency. The "burning feet syndrome" apparently is common in the Far East where vitamin deficiency states are frequent. The etiology seems to be in doubt, some observers having described improvement with the administration of thiamin alone, others with pantothenic acid alone. Bibile et al. [8], in a well controlled study of sixty cases of the "burning feet syndrome," found slightly better response to placebo therapy than with large amounts of both orally and parenterally administered calcium pantothenate. They concluded that the syndrome was not due to pantothenic acid deficiency. Smith and Woodruff [9], as a result of their observations of Japanese prison camps during World War II, concluded that the syndrome was due to B-complex deficiency and was much improved with the use of thiamin alone. Gopalan [10] cured fifty-three patients with the syndrome with amazing rapidity, using 40 mg. calcium pantothenate daily. Krikler's patient exhibited marked burning of the feet but did not respond to the administration of thiamin.

Apparently the burning feet syndrome may manifest various degrees of severity, ranging from mild aching and burning to severe, un-

bearable nocturnal and diurnal burning and tenderness of the feet. Reflex changes and muscular atrophy may or may not be an accompaniment. The response to any type of therapy is usually slow, i.e., two or three months, and in our case was the last symptom to disappear. Since many patients do not seem to respond to vitamin therapy (the present patient received therapeutic doses of multivitamin for a period of one year without appreciable change in symptoms), could it not be that protein depletion *per se* is a major etiologic factor in this distressing symptom? Certainly our case suggests this. Whereas the patient failed to respond to large vitamin intake, the burning feet syndrome slowly disappeared after correction of the protein deficit.

Unfortunately, at the time the patient was in the hospital we did not have available the intestinal biopsy capsule and consequently microscopic examination of the intestinal mucosa was not performed. No attempt to obtain this was made during laparotomy, primarily because we did not believe that we should jeopardize the patient's condition by adding to his surgical morbidity.

It is interesting to speculate as to how the diarrhea may have first started. Studies obtained on intestinal biopsies indicate that the malabsorption state either may precede or result from changes in the intestinal mucosa [*11*]. At the present time it is difficult to say which occurs first. Certainly protein depletion leads to degeneration and alteration of function of the intestinal mucosa [*11*].

The cause of the protein depletion is not apparent in our case. There was nothing indicated by the surgical procedure; no intestinal parasite was found; and no lymphadenopathy, fever or other evidence to suggest Whipple's disease. It appears unlikely that a normally functioning gastroenterostomy would, after ten years, suddenly precipitate such a condition. One might postulate that in the beginning the patient had a severe protracted gastroenteritis which led to protein depletion and this in turn caused added damage to the intestinal mucosa, thereby establishing a vicious cycle. However, should protein depletion occur in such a manner, we might also expect malabsorption of fat. The tests performed on the patient did not indicate any disturbance in fat absorption, although at a previous hospital increased total fat and free fat in the stool were reported (the values for this are not available). (Fig. 2A, p. 849.) In addition, the marked depletion of protein could cause degeneration of the functional capacity of the pancreas, leading to marked exaggeration of the malabsorption state and a further loss of fecal nitrogen. It is unfortunate that fecal nitrogen studies and pancreatic enzyme determinations were not available.

Our case demonstrates rather remarkably the effect of protein depletion on the hormonal system of the body, including the thyroid, pancreas, adrenals, pituitary and gonads. It further demonstrates that even after a prolonged period of depletion, almost two years, the endocrine system seems capable of recovery. This was amply manifested by an increase in 17-ketosteroid excretion, disappearance of abnormal glucose tolerance, reversal of an abnormal Thorn test, subjective and objective improvement in sexual activity, and recovery of secondary sex characteristics. The thyroid apparently remained depressed as measured by the continued diminution of the iodine uptake. A stimulation test with TSH was not performed. The difficulty of differentiating functional hypopituitarism has been adequately pointed out by Perloff et al. [*3*].

Jacobs [*12*] reported loss of libido, loss of hair and testicular atrophy in American prisoners of war in Japan during World War II. He found reversal of these changes forty months after liberation and adequate diet.

Exfoliative dermatitis has been reported to be a frequent manifestation of protein deficiency [*6*]. The exfoliation disappeared in our case shortly after massive administration of plasma.

SUMMARY

A kwashiorkor-like syndrome, associated with "burning feet," is described in a forty-five year old Negro man, in whom, ten years after a gastrectomy for intractable duodenal ulcer, progressively severe diarrhea developed which resulted in pronounced protein depletion, affecting particularly the endocrine glands, with apparent decline in the trophic hormones. After all possible etiologies were considered, the patient was explored surgically and no abnormality was found. In the process of preparing for surgery the patient was given a large quantity of plasma protein, including 20 units of plasma, and apparently as a result of this therapy his severe protein depletion was corrected. Subsequently, the patient rapidly recovered.

The clinical picture was not unlike that described in the syndrome of kwashiorkor, even to the red hair. Eight months after admission to the hospital, and five months after the institution of protein therapy, the patient appears and acts perfectly healthy.

Acknowledgment: We are indebted to Lewis Capland, M.D., consultant in Dermatology to the V. A. Hospital, Coral Gables, Florida, for his suggestion of the diagnosis of kwashiorkor.

REFERENCES

1. Mellinkoff, S. M. Temporary redness of the hair in ulcerative colitis. *Am. J. Dig. Dis.*, 2: 738, 1957.
2. Krosnick, A. and Kalser, M. H. Differential diagnosis of functional hypopituitarism due to malnutrition (idiopathic steatorrhea) and organic pituitary insufficiency in the puerperium. *Metabolism*, 5: 710, 1956.
3. Perloff, W. H., Lasche, E. M., Nodine, J. H., Schneeberg, M. G. and Vieillard, C. B. The starvation state and functional hypopituitarism. *J. A. M. A.*, 155: 1307, 1954.
4. Krikler, D. M. and Schrire, V. "Kwashiorkor" in an adult due to an intestinal blind loop. *Lancet*, 1: 510, 1958.
5. Shaper, A. G. Letter to editor. *Lancet*, 2: 746, 1958.
6. Leitner, Z. A. Postoperative malabsorption. *Lancet*, 2: 504, 1958.
7. Keys, A., Brozek, J., Henschel, A., Mickleson, O. and Taylor, H. L. Biology of Human Starvation. Minneapolis, 1950. University of Minneapolis Press.
8. Bibile, S. W., Lionel, N. D. W., Dunuwille, R. and Perera, G. Pantothenol and the burning feet syndrome. *Brit. J. Nutrition*, 11: 434, 1957.
9. Smith, D. A. and Woodruff, M. F. A. Report on deficiency diseases in Japanese prison camps. *Spec. Rep. Ser. M. Res. Coun., London*, 274: 1, 1951.
10. Gopalan, C. "Burning-feet" syndrome. *Indian M. Gaz.*, 81: 22, 1946.
11. Volwiler, W. Gastrointestinal malabsorptive syndromes. *Am. J. Med.*, 23: 250, 1957.
12. Jacobs, E. C. Effects of starvation on sex hormones in the male. *J. Clin. Endocrinol.*, 8: 227, 1948.

Coronary Embolism*

GERAINT T. GRIFFITH,† M.B.E., F.R.C.P.(C), JOHN TURNER SHARP,‡ M.D. *and*
Chatham, Canada *Hines, Illinois*
DAVID G. GREENE, M.D.
Buffalo, New York

BECAUSE of its infrequency, coronary embolism may be overlooked as a cause of death. In a recent review of the subject Wenger and Bauer [*1*] found that the underlying diseases giving rise to coronary embolism most commonly reported were bacterial endocarditis, intracardiac thrombus, syphilitic aortitis and aortic atherosclerosis. These authors also point out the rarity of the condition, which was found in only 0.06 per cent of 17,469 consecutive autopsies performed at the Mount Sinai Hospital.

The following case is unique in two respects. First, there is no previous record of the electrocardiographic changes in man during and following embolization of a large coronary vessel. Second, this is the first reported fatality due to coronary embolization occurring during cardiac surgery.

CASE HISTORY

B. W., aged twenty-five, had one attack of rheumatic fever when he was eight years old, after which a bruit was noted. He was advised to limit his activities. Eighteen months before this admission to the hospital he noticed dyspnea on exertion. This became progressively more severe, interfering with his normal life. One year previously he had swelling of the ankles which was controlled by the administration of digitalis, mercurial diuretics and a low salt diet. Infrequently he had small hemoptyses.

On examination he was a thin, sallow, white man. Abnormalities were limited to the cardiovascular system. The peripheral pulses were of normal volume. The heart rhythm indicated atrial fibrillation. The jugular venous pressure was normal. The apex beat presented a sustained thrust in the sixth interspace outside the midclavicular line and a diastolic thrill was palpable at this site. A moderate parasternal lift was present. There was no accentuation of the first heart sound. A moderately loud systolic murmur and a rumbling diastolic murmur were audible at the apex. An opening snap was heard by some observers.

Fluoroscopy of the heart revealed enlargement of both atria and the right ventricle. Mitral valve calcification was visible. The electrocardiogram (Fig. 1) confirmed atrial fibrillation and showed right ventricular hypertrophy. Before surgery, combined pressure measurements using a cardiac catheter and double anterior percutaneous heart puncture as described by Greene et al. [*2*] gave the following results: Aorta 95/70 mm. Hg, pulmonary artery 98/49 mm. Hg, left atrium 28/14 mm. Hg, and left ventricle 96/1 mm. Hg. The average gradient across the mitral valve was 13 mm. Hg. The cardiac index was 1.9 L./minute/m^2.

With a gradient across the mitral valve and a left atrial pulse pressure of 14 mm. Hg, commissurotomy was indicated although it was appreciated that a degree of mitral insufficiency was also present.

The left atrial and left ventricular pressures were measured after thoracotomy, together with lead II of the electrocardiogram. (Fig. 2.) A narrowed mitral valve, calcified, thickened and relatively immobile with a moderate regurgitant flow was cut anteriorly without increasing the regurgitant jet. Pressure measurements were repeated. (Fig. 3.) These confirmed the surgeon's impression that the left atrial pressure had been reduced and at this time elevation of the S-T segment was first noted. Because of the severity of the patient's disease it was decided to enlarge the mitral opening further. After this the patient's blood pressure failed to rise from the transient hypotension usually found following temporary obstruction of the mitral valve, and it was thought that the regurgitant jet had been increased. In view of the patient's deteriorated condition no further intracardiac pressure measurements were made. The elevation of the S-T segment noted after the first incision into the valve had increased greatly by this time (Fig. 4 upper) and the T wave had become inverted. Bursts of ventricular tachycardia occurred. (Fig. 4 middle.) The retractors and chest spreaders

* From the Department of Medicine, University of Buffalo School of Medicine and the Buffalo General Hospital, Buffalo, New York.
† Research Fellow of the American Heart Association.
‡ Established Investigator of the American Heart Association.

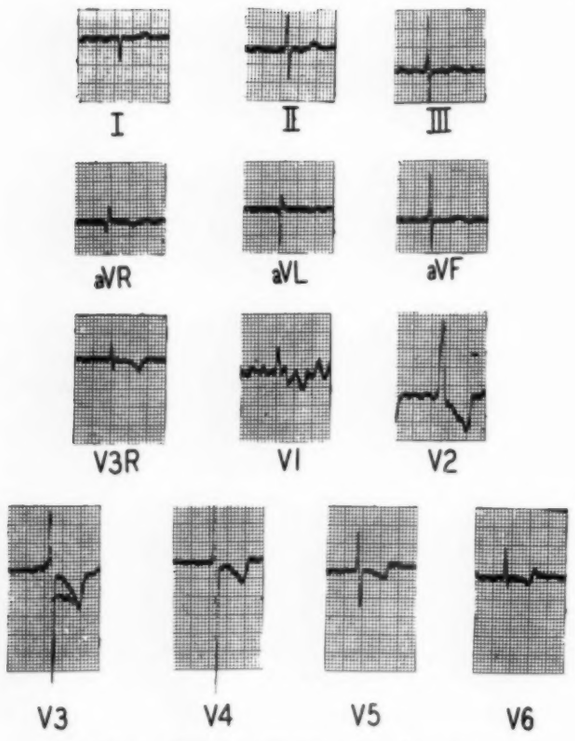


FIG. 1. Preoperative electrocardiogram.

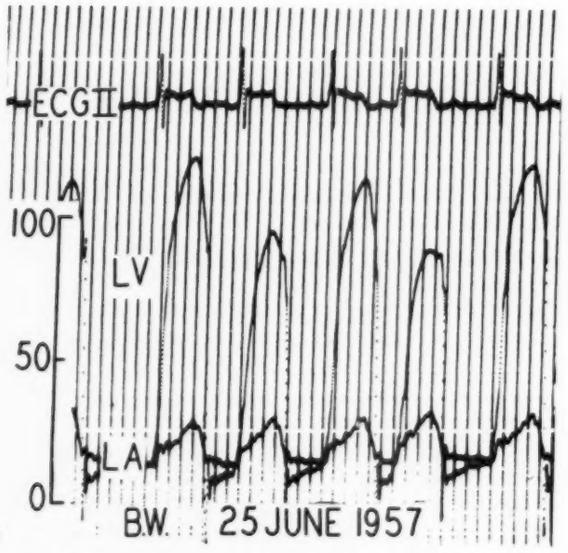


FIG. 3. Pressure tracings of the left atrium and left ventricle after the first incision through the valve. Lead II of the electrocardiogram shows elevation of the S-T segment.

were removed, and the patient was ventilated with pure oxygen. No drugs were employed, and the ventricular tachycardia ceased, the complexes reverting to their previous abnormal shape. (Fig. 4 lower.) During the next half hour the patient was observed, and his condition improved somewhat.

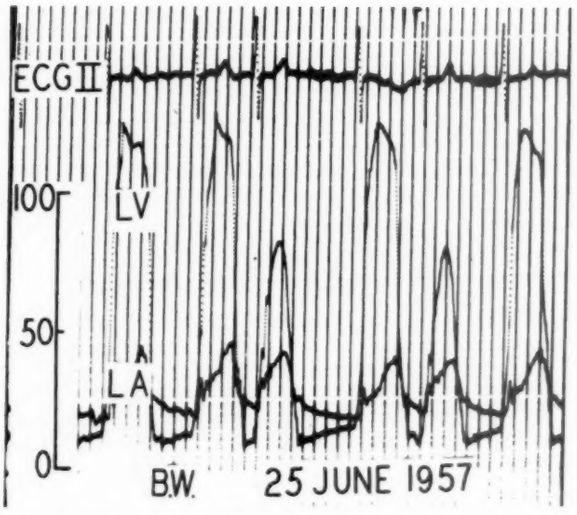


FIG. 2. Left atrial and left ventricular pressure tracings and lead II of the electrocardiogram at thoracotomy immediately before valvotomy.

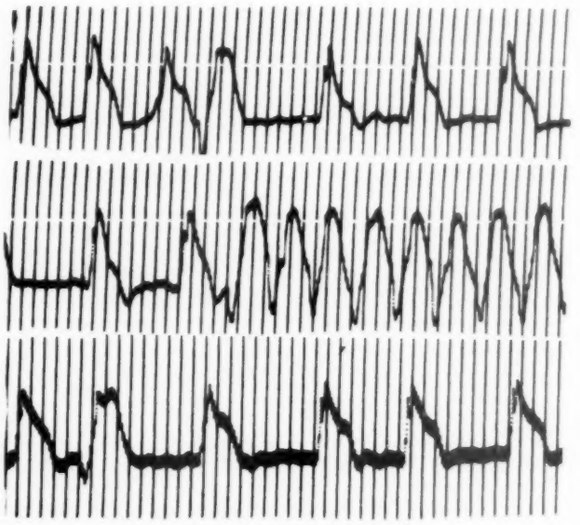


FIG. 4. Lead II of the electrocardiogram after the second incision through the valve. The upper tracing shows the broad complexes with the high S-T take-off followed by ventricular tachycardia (middle) and reversion to the previous complexes (lower).

Although no further minute-to-minute electrocardiographic records are available, it was noted from the oscilloscope that the high S-T take-off from the QRS complex became progressively lower. (Fig. 5.)

The chest wound was repaired and the patient was returned to the ward. Postoperatively the patient's course was marked by restless agitation, mania, hypotension (despite L-norepinephrine therapy) and anuria.

Figure 6 shows an electrocardiogram obtained one hour before the patient's death on the following morning; this was interpreted as showing atrial

FIG. 5. Complexes changing during the half hour following valvotomy showing progressive lowering of the S-T take-off.

fibrillation, right bundle branch block and posterior wall infarction.

At autopsy the lungs showed acute and chronic congestion. The heart weighed 550 gm., and all chambers were dilated. The left atrial appendage was hemorrhagic where the purse string sutures had been placed. The right ventricle was 0.9 cm. thick and the left ventricle 1.5 cm. The mitral valve was thickened and fibrosed, with calcification and rheumatic vegetations. It showed the recent commissurotomy anteriorly. There were areas of hemorrhage in the walls of both ventricles and in the interventricular septum. The left coronary artery was normal. The right coronary artery was occluded 7 cm. from its origin by an embolus composed of calcified material and blood clot, similar to the vegetations on the mitral valve. The embolus was 13 mm. long. The coronary arteries were not atheromatous. Histological examination confirmed that the embolus was composed of calcific material and thrombus. The myocardium showed the classic changes of early infarction.

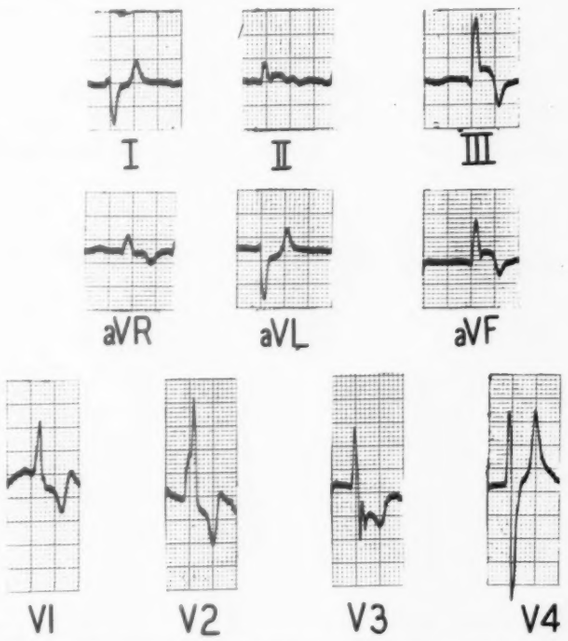


FIG. 6. Electrocardiogram taken one hour before death.

COMMENTS

In a detailed study of the myocardium in fourteen cases of rheumatic aortic and mitral disease, Wigle [*3*] found small calcareous emboli in three. In one of these surgery of the aortic valve had been performed a year previously. In the other two the embolism was spontaneous. Kavanaugh et al. [*4*] also reported a case in which a calcareous embolus was believed to be due to surgery performed a year before. In the case here reported the electrocardiographic changes (elevation of the S-T segment) followed the first incision into the valve. This, combined with the histological changes of early myocardial infarction found at autopsy, made it reasonably certain that the calcareous embolus was thrown off at the time of fracture of the valve. After the embolus serial electrocardiograms initially showed moderate elevation of the S-T segment with upright T waves. This was followed by inversion of the T waves and gross elevation of the S-T segment so that its take-off was close to the peak of the R wave. The sudden myocardial ischemia produced irritable foci which were responsible for the periods of ventricular tachycardia. The embolus had its maximal effect (both clinically and electrocardiographically) after the second incision into the valve. In the half hour following this there was clinical improvement, and the elevations of the ischemic S-T segment became less marked, presumably because the blood supply to the damaged myocardium improved.

Following operation and until the patient's death twenty hours later the clinical picture was that of cardiac infarction with shock, and the electrocardiogram showed changes of posterior wall infarction with right bundle branch block.

The causes of coronary embolism are changing with medical therapy. Five of twelve reported cases of air embolism were due to artificial pneumothorax for tuberculous disease, an accident which will occur less often with the lessened use of this therapy. Better and earlier treatment of bacterial endocarditis should tend to diminish the number of emboli therefrom. By contrast, death from coronary

emboli dislodged from intracardiac surgery may be expected to increase.

SUMMARY

A case of embolism of the right coronary artery with calcareous material from the mitral valve following surgical intervention is reported. Serial electrocardiograms from the onset of the embolus are presented.

ADDENDUM

Since the acceptance of this paper for publication an account of a somewhat similar incident in relation to surgery has appeared [*5*].

REFERENCES

1. Wenger, N. K. and Bauer, S. Coronary embolism. *Am. J. Med.*, 25: 549, 1958.
2. Greene, D. G., Sharp, J. T., Griffith, G. T., Bunnell, I. L. and Macmanus, J. E. Surgical applications of anterior percutaneous left heart puncture. *Surgery*, 43: 1, 1958.
3. Wigle, E. D. Myocardial fibrosis and calcareous emboli in valvular heart disease. *Brit. Heart J.*, 19: 539, 1957.
4. Kavanaugh, G. J., Pruitt, R. D. and Edwards, J. E. Coronary embolism and cystic medial necrosis of the ascending aorta associated with calcific aortic stenosis. *Proc. Staff Meet., Mayo Clin.*, 33: 222, 1958.
5. Winters, W. L., Jr., Lautsch, E. V. and Soloff, L. A. Coronary embolism following repair of a ventricular septal defect. *Circulation*, 21: 77, 1960

Rupture of an Aneurysm of the Sinus of Valsalva*

EUGENE J. LIPPSCHUTZ, M.D. *and* LORING W. WOOD, M.D.
Buffalo, New York

UNTIL recently, aneurysmal dilatation of one of the three sinuses of Valsalva was a diagnosis rarely made before death, and in the majority of instances was recognized only when the pressure gradient across the sac became great enough to produce a rupture into one of the chambers of the heart or less frequently into the pulmonary artery or even into the pericardial spaces. In August 1957, after a thorough search of the literature, Sawyers, Adams and Scott [*1*] reported only forty-seven cases in which the diagnosis of congenital aneurysms of the sinuses of Valsalva was made since the original report of Thurnam in 1840. In this series rupture occurred in thirty-seven. There is a fairly detailed anatomic description of the lesion, derived from autopsy findings, in forty-five of the forty-seven cases. In the other two cases, the patients were still living, having had catheterization studies carried out at the time of the report. In none was there a successful repair of the lesion which ultimately proved fatal. The clinical picture and the course from onset to death was quite characteristic and has been thoroughly described by others.

During the past few years there has been an increasing number of reports describing more or less successful repairs of ruptured congenital aneurysms of the sinuses of Valsalva using varying technics and approaches. The most recent and most successful procedures have been performed under extracorporeal circulation, using an approach through the right atrium, repairing the defect anatomically with either a prosthesis or a suture of the aortic wall to the aortic valvular ring.

This report describes a case which was clinically characteristic of ruptured aneurysm of the sinus of Valsalva, confirmed by right heart catheterization and by retrograde aortography, and successfully repaired using bypass circulation.

CASE REPORT

This twenty-one year old white unmarried girl had been perfectly well with the exception of a traumatic compression fracture of the third and fourth lumbar vertebrae in 1953 with no residual disability. On August 18, 1957, about twenty-four hours after her usual weekend hike in woods and hills and shortly after eating pizza, she had an episode of nausea and retching. The next morning she noticed gradually increasing dyspnea and severe substernal pressure radiating into the back followed by upright orthopnea and palpitation during that night. These symptoms remained severe for one week then gradually subsided leaving mild residual retrosternal pain and dyspnea. She was admitted to the Buffalo General Hospital on August 24, 1957, for further evaluation.

Physical examination revealed an obese slightly dyspneic young woman. The skin color was normal and the nailbeds pink. There was regular sinus rhythm with an apical rate of 112. The blood pressure was 140/70 mm. Hg. There was no thrill, but a harsh grade 4 to and fro murmur, machinery-like in character, was heard best in the first interspace to the right of the sternum. (Fig. 1.) This murmur had not been heard on several previous well documented examinations including a previous admission to this hospital for orthopedic reasons. The lungs were clear and resonant; there were no palpable abdominal organs; femoral pulses were good and no peripheral edema was noted. There was no clinical evidence of engorgement.

A chest roentgenogram showed a cardiothoracic ratio of 14:26 as compared with a ratio of 12:26 in 1955. (Fig. 2.) An electrocardiogram was within normal limits, the sinus rhythm was 110 per minute; P-R interval was 0.12 second; QRS interval was 0.08 second describing a narrow clockwise loop of axis +30 degrees and a T axis of 0 degrees. Precordial transition was at lead V_1 for QRS and lead V_2 for T. There was terminal slurring of the S wave in lead V_1. S-T segments were isoelectric throughout.

* From the Department of Medicine of the Buffalo General Hospital and the University of Buffalo School of Medicine, Buffalo, New York.

FIG. 1. Phonocardiogram.

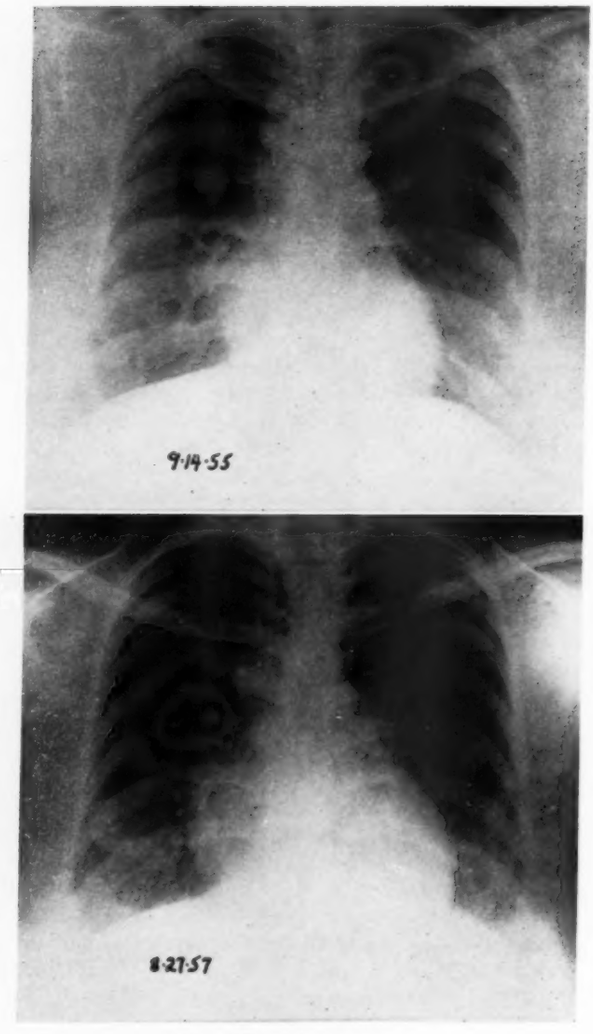


FIG. 2. Roentgenograms.

The laboratory findings were: fasting blood sugar, 91 mg. per cent; blood urea nitrogen, 12 mg. per cent; red blood count, 5,500,000 per cu. mm.; hemoglobin, 16.2 gm. per cent; hematocrit, 44 per cent; sedimentation rate, 100 mm. per hour corrected; leukocytes, 8,000 per cu. mm. with band forms, 8 per cent; segmented forms, 34 per cent; eosinophils, 2 per cent; lymphocytes, 51 per cent; and monocytes, 5 per cent. The urine was acid; its specific gravity was 1.027 with a trace of albumin, no glucose and 5 to 7 white blood cells per high power field. The antistreptolysin titer was 250 units and two blood cultures were reported sterile after five days. The basal metabolic rate was minus 5 per cent on August 30, 1957.

TABLE 1
CARDIAC CATHETERIZATION DATA

	Oxygen Content (Van Slyke) (vol. %)	Oxygen Saturation (Cuvette) (%)	Pressure (mm. Hg)
Left pulmonary artery	11.0	75	.....
Right pulmonary artery	....	77	37.5/18
Right ventricle at the pulmonary valve	12.5	77	37.5/0
Right ventricle at tricuspid valve	....	75	37.5/3
Right atrium at tricuspid valve	....	76	7
Mid right atrium	....	75	7
Superior vena cava	9.5	55	.....
Aorta	14.5	95	112/56

Due to the absence of the Buffalo General Hospital catheterization team, the patient was transferred to the Buffalo Children's Hospital where right heart catheterization and retrograde aortography were performed by Dr. Edward Lambert and his associates. The right heart procedure revealed evidence of a left-to-right shunt at the atrial level, as well as slight pulmonary hypertension. (Table 1.) The systemic pulse pressure was 55 mm. Hg. Another catheter was introduced retrograde through the aortic arch via the brachial artery. As the tip approached the aortic valve it entered a low pressure chamber, presumably the right atrium. The tip was repositioned within the aortic root and 40 cc. of 50 per cent Hypaque® solution was injected under 250 pounds of pressure. Pressure readings before and after injection were the same as those of the ascending aorta. Examination of the radiographs revealed that the cardiac catheter had advanced either into or through the fistulous tract during injection, producing opacification of the right atrium and the pulmonary artery. (Fig. 3.) Although there was no demonstration of the aortic valve region, the procedure was considered indirect confirmation of a ruptured sinus of Valsalva into the right atrium.

Upon the basis of this information the patient was scheduled for surgery, to be performed by Dr. Dwight C. McGoon at the Mayo Clinic in Rochester, Minnesota. On January 22, 1958, some five months after the onset of symptoms, the chest was entered via bilateral anterior thoracotomy incision. The heart was markedly enlarged both to the right and to the left. The pulmonary artery was considerably dilated, and its sinuses were markedly prominent. There was a prominent thrill over the right atrium anteriorly near the tricuspid ring. Pressure measurements

revealed a systolic pressure of 40 mm. Hg in the right ventricle when the systemic pressure was 110 mm. Hg. Extracorporeal circulation was instituted by cannulating the left subclavian artery with a 5.4 mm. cannula and cannulating the venae cavae through the atrial appendage. After instituting extracorporeal circulation, the aorta was cross clamped and the right atrium was opened. The aneurysm could be seen as a cylindrical sac projecting into the right atrium with an opening at its end measuring 5 mm. in diameter. This sac was clamped with a hemostat and the aortic clamp was then released. After one minute of perfusion of the coronary arteries, asystole was induced by electrolyte perfusion and was maintained for twenty-four minutes. The thin-walled aneurysm was excised to its base, and the aortic wall above and the aortic ring below were identified. These were approximated by several interrupted sutures of No. 3-0 silk. A prosthesis was not used. The aortic clamp was again released and the repair was free of leakage. The incision in the atrium was then closed and as the bypass circulation was being removed there was a period of two to three minutes of rapid ventricular fibrillation which subsided spontaneously. Pressures were again measured with little change in the right ventricle or atrium. In the early postoperative period there was considerable respiratory distress leading to the introduction of a tracheostomy about twenty-four hours after surgery. Following this the patient improved rapidly and was dismissed from the hospital twelve days after surgery. At this time her exercise tolerance was much improved and her heart was quiet with no murmurs. The digitalis, which had been administered for several weeks prior to surgery, was discontinued and she was sent home with the advice to restrict both her sodium intake and her weight gain. When she last communicated with us in December 1958 she stated that she felt generally well, had no restriction of her activities, had married and subsequently carried through a full term pregnancy and delivery of a healthy baby without experiencing any cardiac difficulty.

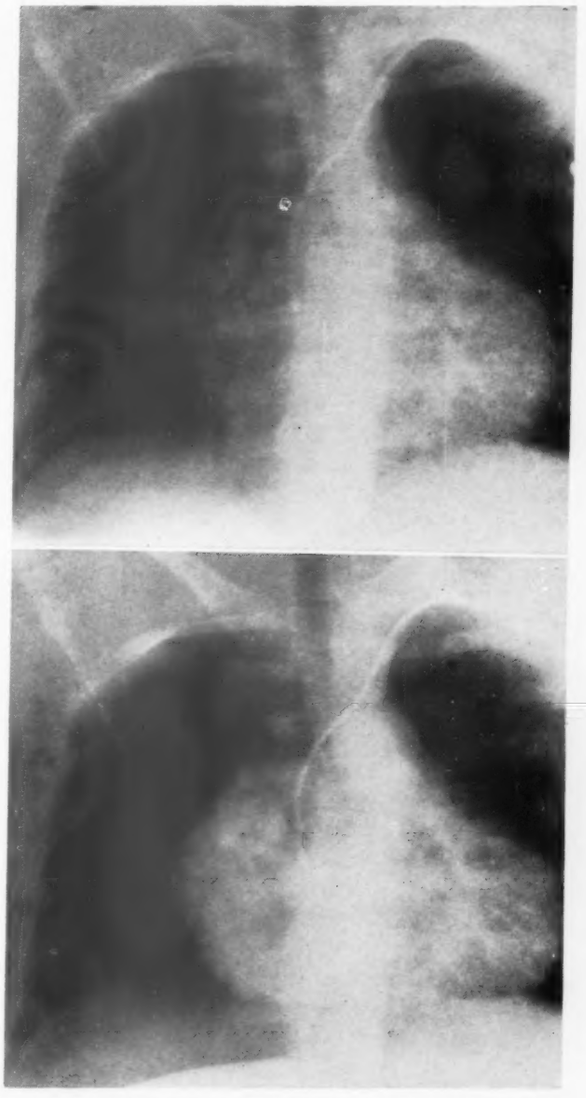


FIG. 3. Roentgenograms before and after injection of dye.

COMMENTS

This case serves as a dramatic illustration of the recent shift in emphasis on the antemortem diagnosis of ruptured aneurysm of a sinus of Valsalva from that of academic desirability to that of prognostic urgency. This stems from recent repeated demonstrations of successful surgical correction [*7,9*] of a lesion which is otherwise invariably fatal over a period of seven days to seventeen years [*6*]. The surgical feasibility is enhanced by several factors. The relative ease of diagnosis is initially suggested by the characteristic clinical picture of sudden chest pain, dyspnea, congestive failure in a previously healthy person and the sudden appearance of a "machinery-like" murmur over the base of the heart. This is confirmed through the specific information gained by right heart catheterization, retrograde aortography and more recently by selective dye dilution methods [*11*] which are of great value to the cardiologist and the surgeon preoperatively. The characteristic latent period following the initial onset of symptoms, during which the patient is in reasonably good condition and able to withstand the major surgery involved, is important.

This patient satisfied all the criteria, with the exception of the sex distribution which was predominantly male, and of the clinical evidence of both aortic and tricuspid valvular regurgitation

which is frequently seen in the later stages [*1,6*] of ruptured aneurysm of a sinus of Valsalva. Although the exact anatomic location of her aneurysm was not demonstrated by aortography, the fact that it ruptured into the right atrium places it statistically in the posterior or non-coronary sinus. In the review of forty-seven cases by Sawyers, Adams and Scott [*1*], 30 per cent occurred in the non-coronary sinus and all ruptured into the right atrium. The remaining 70 per cent were in the right coronary sinus and only three of these ruptured into the right atrium, while nineteen ruptured into the right ventricle and one each into the left ventricle, pulmonary artery and pericardium. In another review of twenty-three cases, Oram and East found that seven aneurysms occurred in the non-coronary sinus, all of which ruptured into the right atrium [*6*].

Generally speaking, the occurrence of aneurysm of the sinus of Valsalva is rare and comes to clinical attention only when rupture occurs. Jones and Langley [*10*], quoting Smith (1914), reported an incidence of seven sinus aneurysms in 8,138 general autopsy examinations. However, Steinberg [*8*] stated that in a series of 3,000 angiocardiograms over an eleven-year period, there were twenty-five unruptured aneurysms of the aortic sinus. It must be said that this does not reflect the true incidence of the lesion since all twenty-five occurred in association with other lesions which were the basic reasons for angiocardiography. Of the fifteen congenital aneurysms, six were associated with coarctation, one with pseudo-coarctation, one with aortic and mitral valvular disease, and seven with arachnodactyly. Of the acquired aneurysms, all were associated with aortic incompetence. The number of unsuspected and unphotographed lesions remains an imponderable quantity, and although it has been reported that unruptured aneurysms may produce chest pain and cardiac murmurs [*6,12*], the correlation is inconsistent and difficult to confirm. Rupture remains the only reliable and significant indication of the presence of an aneurysm, particularly those of congenital origin, in the absence of associated lesions.

The basic etiology of the congenital type of aneurysm has been well demonstrated by Burchell and Edwards [*3,4*] as a defect between the aortic root and the heart manifested as a failure of union of the elastic media of the aortic wall above with the annulus fibrosus of the aortic valve ring below. The same type of defect may occur below the valve ring giving rise to a ventricular septal defect. These two lesions are occasionally seen in combination [*4,10*]. The events leading to the eventual rupture of the weakened section of aortic wall appear to be: (1) the continuous stress imposed by high pressures within the aortic lumen, and (2) gradual protrusion into an adjacent low pressure chamber as an aneurysmal sac [*5*]. Consequently, the locus of protrusion and eventual rupture is predominantly into the right side of the heart, the specific chamber thereof depending upon which sinus and which portion of that sinus is involved. Surgical consideration is presently concerned primarily with aneurysms of congenital origin since those lesions secondary to syphilitic or bacterial invasion of the aortic wall are not generally susceptible to current technics of surgical repair.

The most logical approach to repair is to bridge the defect either by direct suture or by prosthesis [*3*], and the best surgical approach appears to be via the right atrium or the right ventricle, depending on the location of the rupture. Mattingly et al. [*7*] report the successful use of a prosthetic tampon to occlude the fistulous tract under hypothermia using hydraulic formulas to estimate the diameter of the fistula prior to surgery. The advantage of this prosthetic closure is said to be that of obliteration of the defect without tension, although it would be facilitated by the use of extracorporeal circulation rather than hypothermia. McGoon, Edwards and Kirklin [*9*] have reported the use of a polyvinyl sponge prosthesis in the successful repair of a ruptured aneurysm of a sinus of Valsalva in combination with a ventricular septal defect, using bypass circulation.

SUMMARY

A case is presented in which a clinical diagnosis of ruptured aneurysm of a sinus of Valsalva was confirmed both by right heart catheterization and by retrograde aortography. Successful repair was effected using a transatrial approach, bypass circulation and direct suture of the defect. A brief discussion points out the urgency of early diagnosis in view of the recent employment of successful surgical technics.

REFERENCES

1. Sawyers, J. L., Adams, J. E. and Scott, H. W., Jr. A method of surgical repair for ruptured aortic

sinus aneurysms with aortic-atrial fistula. *South. M. J.*, 50: 1075, 1957.

2. Sawyers, J. L., Adams, J. E. and Scott, H. W., Jr. Surgical treatment for aneurysms of aortic sinus with aortic-atrial fistula. *Surgery*, 41: 1, 1957.
3. Edwards, J. E. and Burchell, H. B. Specimen exhibiting the essential lesion in aneurysm of the aortic sinus. *Proc. Staff Meet., Mayo Clin.*, 31: 407, 1956.
4. Edwards, J. E. and Burchell, H. B. The pathological anatomy of deficiencies between the aortic root and the heart, including aortic sinus aneurysms. *Thorax*, 12: 125, 1957.
5. Lillihei, C. W., Stanley, P. and Varco, R. L. Surgical treatment of ruptured aneurysms of the sinus of Valsalva. *Ann. Surg.*, 146: 459, 1957.
6. Oram, S. and East, T. Rupture of aneurysm of aortic sinus (of Valsalva) into the right side of the heart. *Brit. Heart J.*, 18: 541, 1955.
7. Morrow, A. G., Baker, R. R., Hanson, H. E. and Mattingly, T. W. Successful surgical repair of a ruptured aneurysm of the sinus of Valsalva. *Circulation*, 16: 533, 1957.
8. Glenn, F., Stewart, H., Engle, M. A., Lukas, D. S., Artusio, J., Steinberg, I. S. and Holswade, G. R. Coarctation of aorta complicated by bacterial endocarditis and an aneurysm of the sinus of Valsalva. *Circulation*, 17: 432, 1958.
9. McGoon, D. C., Edwards, J. E. and Kirklin, J. W. Surgical treatment of ruptured aneurysm of aorta sinus. *Ann. Surg.*, 147: 387, 1958.
10. Jones, M. and Langley, F. A. Aortic sinus aneurysms. *Brit. Heart J.*, 11: 325, 1949.
11. Semer, H. J. and Brandenburg, R. O. Demonstration of site of rupture of aortic sinus aneurysm. *Proc. Staff Meet., Mayo Clin.*, 33: 604, 1958.
12. Steinberg, I. and Finby, N. Clinical manifestations of the unperforated aortic sinus aneurysm. *Circulation*, 14: 115, 1956.
13. Smith, W. A. Quoted by Jones and Langley. *J. A. M. A.*, 62: 1878, 1914.

Maffucci's Syndrome (Dyschondroplasia with Hemangiomas)*

JEROME L. JOHNSON, M.D., JAMES R. WEBSTER, JR., M.D. *and* H. IVAN SIPPY, M.D.
Chicago, Illinois

MAFFUCCI'S syndrome (dyschondroplasia with hemangiomas) is a rare occurrence. Of forty-two cases reported since the original description in 1881 [*1*], only twenty have been recorded in the past fifteen years. Seven of the forty-two patients were examined at autopsy. We recently observed a patient suffering from this disorder who is herein described.

CASE REPORT

A fifty-seven year old white broker was admitted to the Chicago Wesley Memorial Hospital for evaluation of anorexia, nausea and vomiting. The history revealed that he was normal at birth and that the first three years of his life were uneventful. Between the ages of three and thirteen he sustained nine fractures of his left leg. Each was produced by a minor trauma and healed well; however, a residual varus deformity persisted. At age thirty-three he had a left mid-thigh amputation because of pain in his left ankle. Subsequently he was told that a malignancy had been found in one toe. His progress was uneventful until the age of fifty, at which time he fractured his right wrist. The fracture healed satisfactorily. At age fifty-five he fractured his right forearm. This break also healed well.

Throughout his life he had had multiple swellings on his left hand, many of which spontaneously disappeared. During his fifty-sixth year he was afflicted with a red, painful swelling of the third finger of his left hand and, because of progressive growth and changes evident on roentgenograms which were suggestive of malignancy, an amputation of the entire digit was performed in another institution. The pathologic diagnosis was chondrosarcoma. Approximately one year later his left mid-thigh was operated on again also at another institution, and a fibrosarcoma was found. Following this operation the patient returned to work for five months. Three weeks prior to his final admission, he began experiencing anorexia, nausea and vomiting, and shortly thereafter noted dark urine and light stools. Between the onset of these symptoms and his hospitalization he had lost 15 pounds. He had also had several attacks of renal colic, which had subsided with the passage of the offending stone.

The patient stated that his two sisters were living and well, and that his mother had died of carcinoma of the colon. No other members of his family were known to have musculoskeletal disorders.

On admission to the hospital the patient appeared jaundiced and chronically ill. The temperature was 101°F., blood pressure 130/70 mm. Hg, respirations 22 per minute, and the pulse was 120 and regular. Examination of the head, eyes, ears, nose and throat was within normal limits except that the sclera were icteric. There was no significant lymphadenopathy. The chest was clear to percussion, palpation and auscultation. The heart was not thought to be enlarged. A soft, grade 2 apical systolic murmur was present. The abdomen was distended, and the liver edge, palpable 4 cm. below the right costal margin, was firm and nodular. A fluid wave and shifting dullness were present. The skin was warm and dry. A large venous hemangioma, measuring 13 cm. in the greatest dimension, was present on the left anterior side of the chest. There were also multiple hemangiomas on the dorsum of the left hand.

Examination of the musculoskeletal system revealed many deformities. The right humerus, radius, and ulna were shortened and bowed. The left humerus, radius and ulna also were shortened, bowed and somewhat thickened. There were multiple soft, freely movable masses on the dorsum of the left hand. The largest one was ovoid, and it measured 4 by 3 by 2 cm. The first digit of the left hand was thickened and abnormally bowed, and the third digit was missing. At the junction of the middle and distal phalanges of the fourth finger there was a large, hard, slightly tender mass which seemed to originate in the phalanges. The remnant of the left leg measured 11 cm. in length, and the amputation scar was well healed. On gross examination, the right leg was normal.

Laboratory studies revealed a hematocrit of 42 per cent, a white blood cell count of 22,700 per cu. mm. with a differential count which showed a shift to the left. The fasting blood sugar was 110 mg. per cent, blood urea nitrogen 14 mg. per cent. Serum total protein concentration was 5.5 gm. per cent, albumin

* From the Department of Medicine, Chicago Wesley Memorial Hospital, Chicago, Illinois.

FIG. 1. Roentgenogram of the left arm.

FIG. 2. Roentgenogram of the hands.

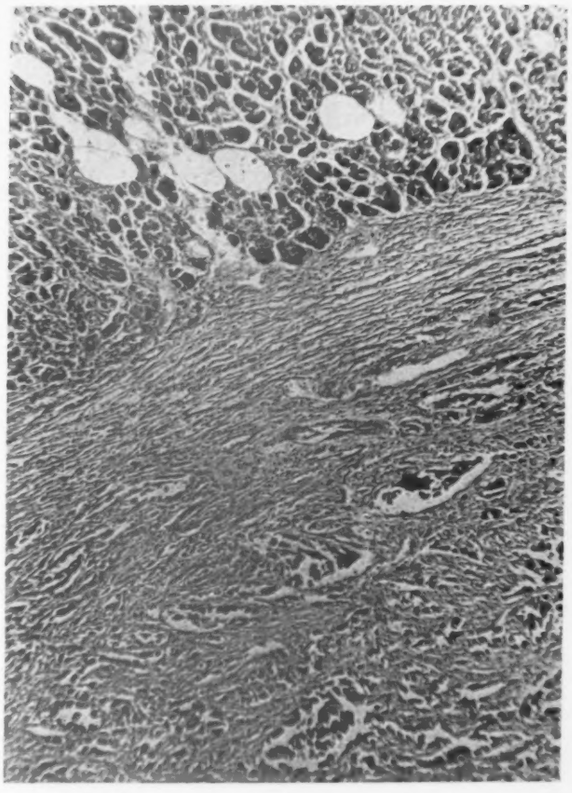


FIG. 3. Photomicrograph of pancreas showing normal tissue (top) and tumor.

2.6 and globulin 2.9 gm. per cent, alkaline phosphatase 17.6 Bodansky units, serum transaminase 240 units, and the serum total bilirubin was 5 mg. with a biphasic reaction.

Roentgenograms of the skeleton showed bowing and shortening of all the long bones of both arms. In addition, the left radius and ulna showed changes which were interpreted as typical of dyschondroplasia. (Fig. 1.) The carpal bones of the left hand appeared to contain multiple enchondromas. The bony protuberance on the fourth finger of the left hand had the appearance of a chondroma. Multiple phleboliths were seen in the soft tissues of the distal portion of the left forearm, in the left hand, and over the left side of the chest. All of these were located in the regions of the patient's hemangiomas. (Fig. 2.) These findings were interpreted as consistent with the diagnosis of Maffucci's syndrome.

A diagnostic paracentesis, was performed on the second day, and 2,400 cc. of blood-tinged ascitic fluid, in which tumor cells were identified, were removed. The patient's condition deteriorated rapidly in spite of supportive therapy, and he died on his eighth hospital day.

At autopsy there were 2,000 cc. of yellowish red intra-abdominal ascitic fluid. The right and left lungs together weighed 740 gm. and contained multiple discrete tumor masses, which were present also on the pleural surfaces. These were firm, grayish white, and measured up to 3 by 2 by 2 cm. The liver weighed 3,200 gm. and contained similar tumor accumulations in both the right and left lobes; the right lobe was almost entirely replaced by tumor. In the head of the pancreas there was a single 0.8 cm. nodule which was tan, firm and poorly demarcated from the surrounding

tissue. The periportal lymph nodes were matted together, firm, and filled with pale gray tumor. Tumor implants were present on the surface of the peritonium throughout the abdomen. The heart, spleen, gastrointestinal and genitourinary tracts appeared normal. Permission was not granted for examination of the head and neck.

Microscopic sections of the nodule in the pancreas revealed a poorly differentiated adenocarcinoma of that organ (Fig. 3), with metastatic lesions in the lungs, liver and lymph nodes, and direct spread to the peritoneum. Sections of bone taken from the left humerus showed areas of dyschondroplasia. Sections of skin taken from the left anterior wall of the chest revealed a vascular malformation compatible with the clinical diagnosis of hemangioma.

COMMENTS

This would appear to be a rather typical case of Maffucci's syndrome. These patients are usually normal at birth. The deformities of cartilage and bone and the vascular malformations, become apparent in childhood. Any portion of the skeleton may be involved and the lesions are asymmetrically distributed. Remission of the condition is not unusual in early adult life. It does not appear to be a familial disease, and occurs in both sexes.

Recognition is not difficult if the syndrome is remembered. Radiography may strikingly demonstrate calcified phleboliths within the vascular abnormalities, in addition to the characteristic bony deformities.

Malignant tumors are not uncommonly the cause of death in these patients. Chondrosarcoma has developed in seven patients, and four others have had cancers of various origin. The patient described herein had two sarcomas removed only to die of another, unrelated malignancy, carcinoma of the pancreas. The fact that this man lived an almost normal life span and was productively engaged until shortly before his death is worthy of consideration.

This disorder has been extensively reviewed and the existing knowledge summarized by Carlton [*2*] and Bean [*3,4*]. The syndrome is probably a congenital mesodermal dysplasia which is not inherited but due to multiple defects in a single gene.

SUMMARY

A case of Maffucci's syndrome (dyschondroplasia with hemangiomas) is presented. The patient's history revealed that two sarcomas had been successfully removed. However, the autopsy findings showed that carcinoma of the pancreas with widespread metastasis was the cause of death.

REFERENCES

1. Maffucci, A. Di un caso di encondroma ed angimo multiplo. *Movimento Medico-Chriurico*, 3: 399, 1881. Cited by Carlton et al. [*2*].
2. Carlton, A., Elkington, J., Greenfield, J. and Robb-Smith, A. Maffucci's syndrome (dychondroplasia with hemangiomata). *Quart. J. Med.*, N.S., 11: 203, 1942.
3. Bean, W. B. Dyschondroplasia and hemangiomata (Maffucci's syndrome). I. *Arch. Int. Med.*, 95: 767, 1955.
4. Bean, W. B. Dyschondroplasia and hemangiomata (Maffucci's syndrome). II. *Arch. Int. Med.*, 102: 544, 1958.

HOW WOULD YOU DEFINE *the ideal diuretic?*

The qualities to be sought in an ideal diuretic have been listed[1] as follows:

"(1) Sustained rather than abrupt violent action.

(2) Reduced capacity for electrolyte upheaval.

(3) Convenience of administration; self-administration (oral, rectal, or subcutaneous).

(4) Decreased toxicity (systemic and local irritant action, especially of the gastrointestinal tract).

(5) Effectiveness where others are ineffectual or contra-indicated.

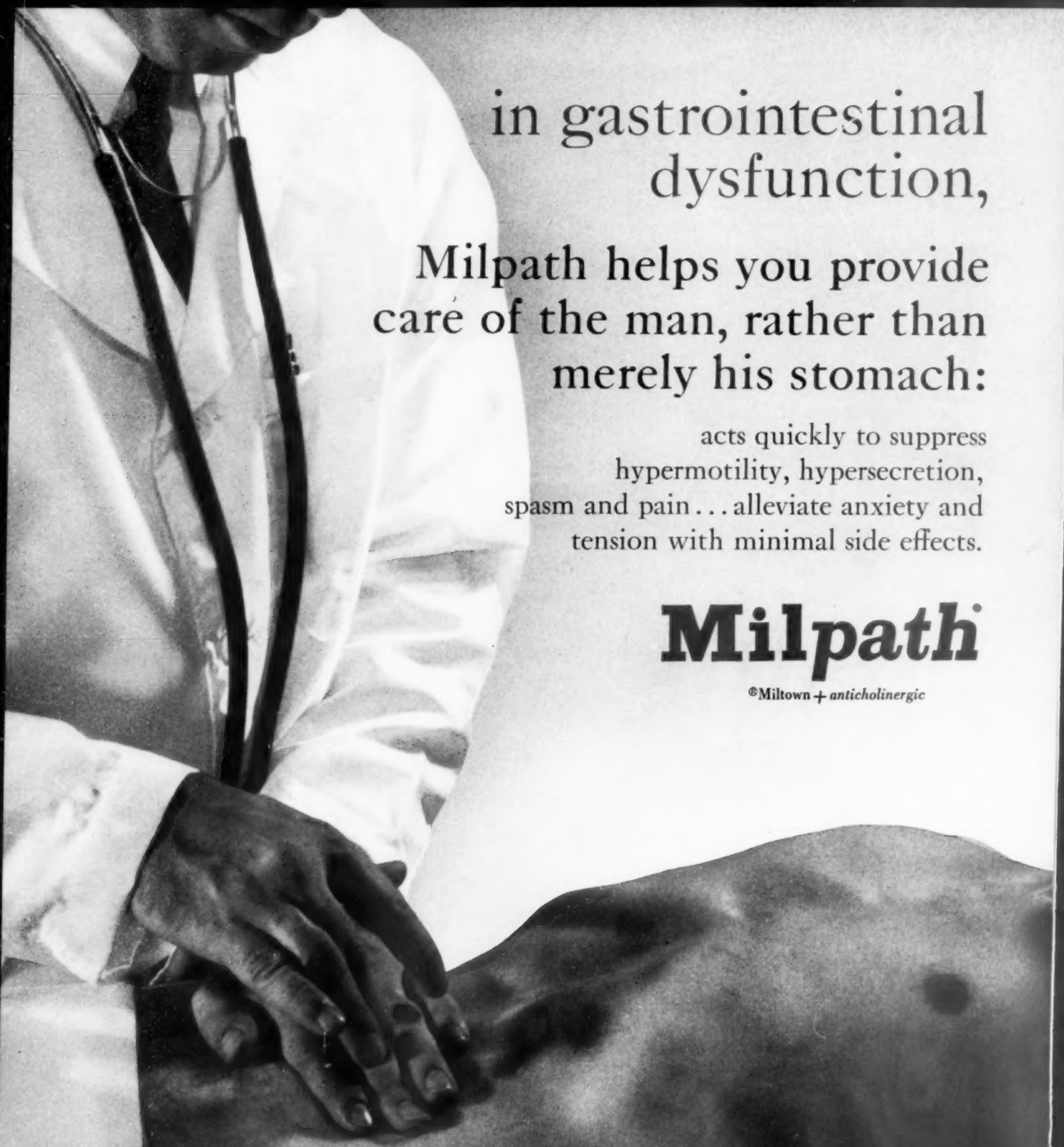
(6) Applicability in cases with a history of allergic reaction to other diuretics."

It would be rash to term any drug ideal. It is safe to say, however, that Aldactone very closely approximates these six requirements. Most importantly, Aldactone often relieves edema and ascites resistant or not optimally responsive to conventional diuretics. By blocking the *action* of the potent salt-retaining hormone, aldosterone, Aldactone, used alone or in synergistic combination with other potent agents, establishes satisfactory diuresis in most edematous patients who have heretofore been considered beyond the help of medical management. Aldactone offers a fundamentally new approach to the control of edema in such disorders as congestive heart failure, hepatic cirrhosis, the nephrotic syndrome and idiopathic edema.

ALDACTONE™ is supplied as compression-coated yellow tablets of 100 mg.
(brand of spironolactone)

1. Modell, W.: Am. J. Cardiol. *3*:139 (Feb.) 1959.

Research in the Service of Medicine G. D. SEARLE & CO.



in gastrointestinal dysfunction,

Milpath helps you provide care of the man, rather than merely his stomach:

acts quickly to suppress hypermotility, hypersecretion, spasm and pain . . . alleviate anxiety and tension with minimal side effects.

Milpath®

®Miltown + *anticholinergic*

AVAILABLE IN TWO POTENCIES

MILPATH-400—Yellow, scored tablets of 400 mg. Miltown (meprobamate) and 25 mg. tridihexethyl chloride. Bottle of 50.

Dosage: 1 tablet t.i.d. at mealtime and 2 at bedtime.

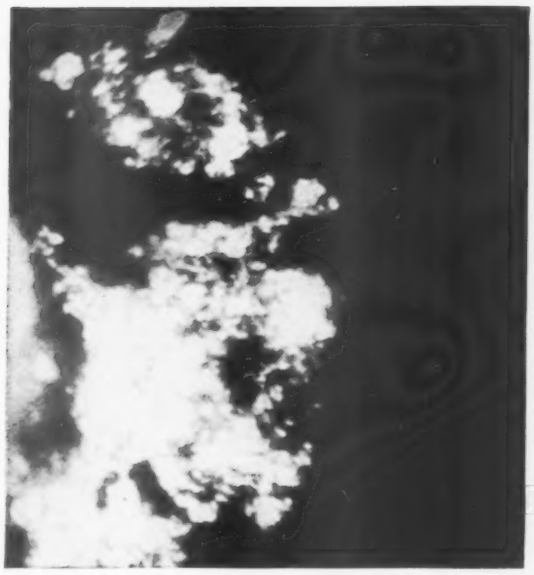
MILPATH-200—Yellow, coated tablets of 200 mg. Miltown (meprobamate) and 25 mg. tridihexethyl chloride. Bottle of 50.

Dosage: 1 or 2 tablets t.i.d. at mealtime and 2 at bedtime.

WALLACE LABORATORIES *New Brunswick, N.J.*

1014

Clarin* can do this for your postcoronary patients



WITHOUT CLARIN, turbid blood serum five hours after a fat meal: This unretouched dark-field photomicrograph (2500X) shows potentially hazardous fat concentrations circulating in the blood stream of a patient after a standard fat meal.

WITH CLARIN, clear blood serum five hours after a fat meal: After eating a standard fat meal as at left, the same patient has taken one sublingual Clarin tablet. Note marked clearing effect and reduction in massive fat concentrations in this unretouched photomicrograph (2500X).

CLARIN is sublingual heparin potassium. One mint-flavored tablet taken after each meal effectively "causes a marked clarification of postprandial lipemic serum."[1] Clarin facilitates the normal physiologic breakdown of fats, with no effects on the blood-clotting mechanism.[1] It therefore provides important benefits for your postcoronary patients.

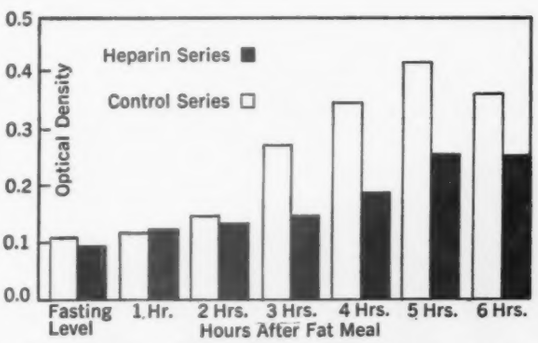
Indication: For the management of hyperlipemia associated with atherosclerosis.

Dosage: After each meal, hold one tablet under the tongue until dissolved.

Supplied: In bottles of 50 pink, sublingual tablets, each containing 1500 I.U. heparin potassium.

1. Fuller, H. L.: Angiology 9:311 (Oct.) 1958.

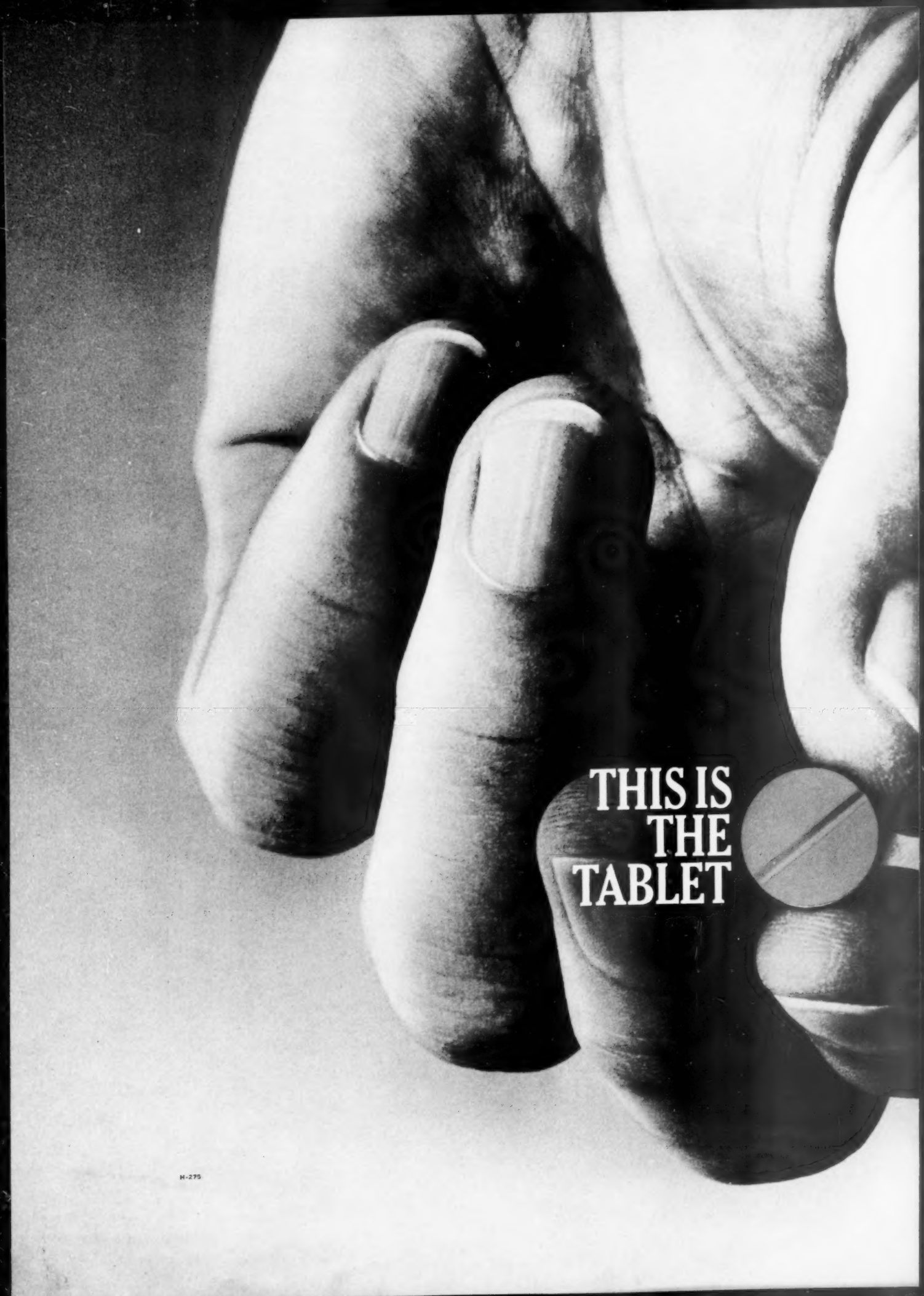
2. Shaftel, H. E., and Selman, D.: Angiology *10*:131 (June) 1959.



Average serum optical density in 36 patients after fat meal with and without sublingual heparin.[2]

*Registered trade mark. Patent applied for.

Thos. Leeming & Co., Inc. New York 17, N. Y.


THIS IS
THE
TABLET
H-275

ALPEN is the oral penicillin that provides, on a fasting stomach, peak antibiotic blood levels approximately twice as high as oral potassium penicillin V... and significantly higher than I. M. penicillin G.

Some strains of staphylococci resistant to other penicillins exhibit in vitro sensitivity to potassium phenethicillin.

ALPEN has greater freedom from the G. I. sequelae (overgrowth of resistant flora) sometimes observed with broad spectrum -mycins.

ALPEN gives much higher antibiotic levels within the first hour of ingestion by the well-tolerated oral route.

WHEN TO USE ALPEN Recommended in the treatment of infections caused by pneumococci, streptococci, gonococci, corynebacteria, and penicillin-sensitive staphylococci.

HOW TO USE ALPEN Depending on the severity of the infection, 125 mg. (200,000 units) or 250 mg. (400,000 units) three times daily may be used. In more severe or stubborn infections, a dosage of 500 mg. (800,000 units) t.i.d. may be employed. In beta hemolytic streptococcal infections, treatment should be continued for at least ten days.

PRECAUTIONS The usual precautions in the administration of oral penicillin should be observed. For further details see package literature.

Tablets: 125 mg. and 250 mg., bottles of 25 and 100. Powder for Oral Solution (lemon-lime flavored), 1.5 Gm. bottle (125 mg. per 5 cc. teaspoonful).

this is the tablet that gives higher peak antibiotic blood levels

HIGHER THAN I. M. PENICILLIN G
HIGHER THAN POTASSIUM PENICILLIN V

ALPEN

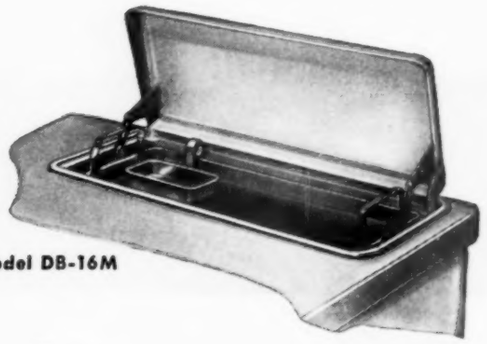
ALPEN™—potassium phenethicillin

Schering

NOW!
A Modern Centralized
STERILE SUPPLY
for Offices and Clinics . . .

Amsco

DOUBLE Cabinet Sterilizer . . .



Model DB-16M

Compact, convenient and easy to operate, the new Amsco Double Cabinet Sterilizer features a handsome double cabinet and utility drawer with ample storage space for instruments and supplies—*and a built-in automatically burn-out proof* **Office Instrument Sterilizer.** The cabinet is also available with a solid top without the recessed boiling-type sterilizer.

The large formica counter provides ample room for work space, while the roomy cabinet and drawers are safe for storage of sterile supplies . . . ready for instant use. The efficient, recessed type A-416S non-pressure sterilizer is fabricated entirely of stainless steel, insulated construction with two trays—one for instruments, the other for needles.

One of the ten popular colors available with the DB-16M will blend perfectly with *your* office decor. A single cabinet with the same mechanical features is also available.

Amsco's large Office Pressure-Steam Sterilizer, 613-R Dynaclave, or 8" Square Autoclave, Cat. No. 8816, can be conveniently located on work counter of the double cabinet.

AMERICAN STERILIZER
ERIE • PENNSYLVANIA

* *See the new Double Cabinet Sterilizers at your authorized Amsco dealer or write for Bulletin DC-404*

first choice for MIGRAINE
recurrent, throbbing "sick" headaches...

CAFERGOT®

When the headache is associated with nervous tension and G.I. disturbance:

CAFERGOT P-B TABLETS: ergotamine tartrate 1 mg., caffeine 100 mg., Bellafoline 0.125 mg., pentobarbital sodium 30 mg. Dosage: 2 at first sign of attack; if needed, 1 additional tablet every ½ hour until relieved (maximum 6 per attack).

CAFERGOT P-B SUPPOSITORIES: ergotamine tartrate 2 mg., caffeine 100 mg., Bellafoline 0.25 mg., pentobarbital sodium 60 mg. Dosage: 1 as early as possible in attack; second in 1 hour, if needed (maximum 2 per attack).

CAFERGOT TABLETS: ergotamine tartrate 1 mg., caffeine 100 mg. Dosage: same as Cafergot P-B Tablets.

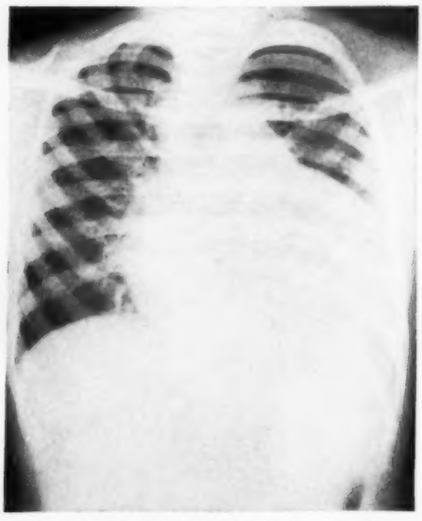
CAFERGOT SUPPOSITORIES: ergotamine tartrate 2 mg., caffeine 100 mg. Dosage: same as Cafergot P-B Suppositories.

For a new brochure on Migraine and Tension Headaches, reviewing clinical reports on diagnosis and therapy, write: Sandoz, Hanover, N. J.

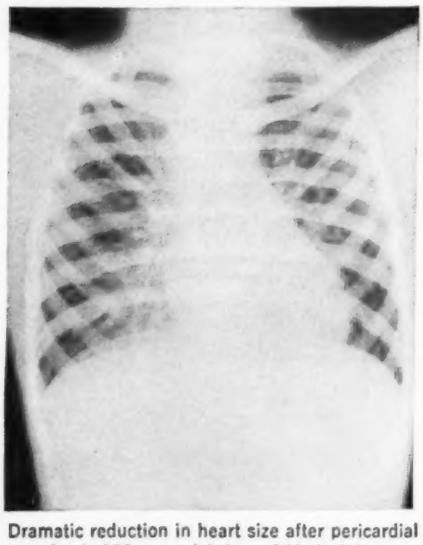
SANDOZ

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN Upjohn

Cardiac damage reduced in acute rheumatic fever



Pericarditis with pericardial effusion; roentgenogram before therapy.



Dramatic reduction in heart size after pericardial tap of only 150 cc. and 6 days of Medrol therapy.

X-rays courtesy of Lorin E. Ainger, M.D.

In 243 patients hospitalized for acute rheumatic fever, *high-dosage corticotherapy* led to regression or disappearance of significant murmurs at least twice as often as did management with salicylate, small doses of steroid, or no medication. *Early initiation of therapy* increased the percentage of patients showing cardiac improvement.[1]

there is only one methylprednisolone, and that is

Medrol*

the corticosteroid that hits the disease, but spares the patient

Supplied: As 4 mg. tablets in bottles of 30, 100 and 500; as 2 mg. tablets in bottles of 30 and 100; and as 16 mg. tablets in bottles of 50.

*TRADEMARK, REG. U. S. PAT. OFF.—METHYLPREDNISOLONE, UPJOHN

1. Massell, B. F.: Paper presented at A Symposium on Steroid Therapy, Chicago, Ill., May 15-16, 1959.

she calls it "nervous indigestion"

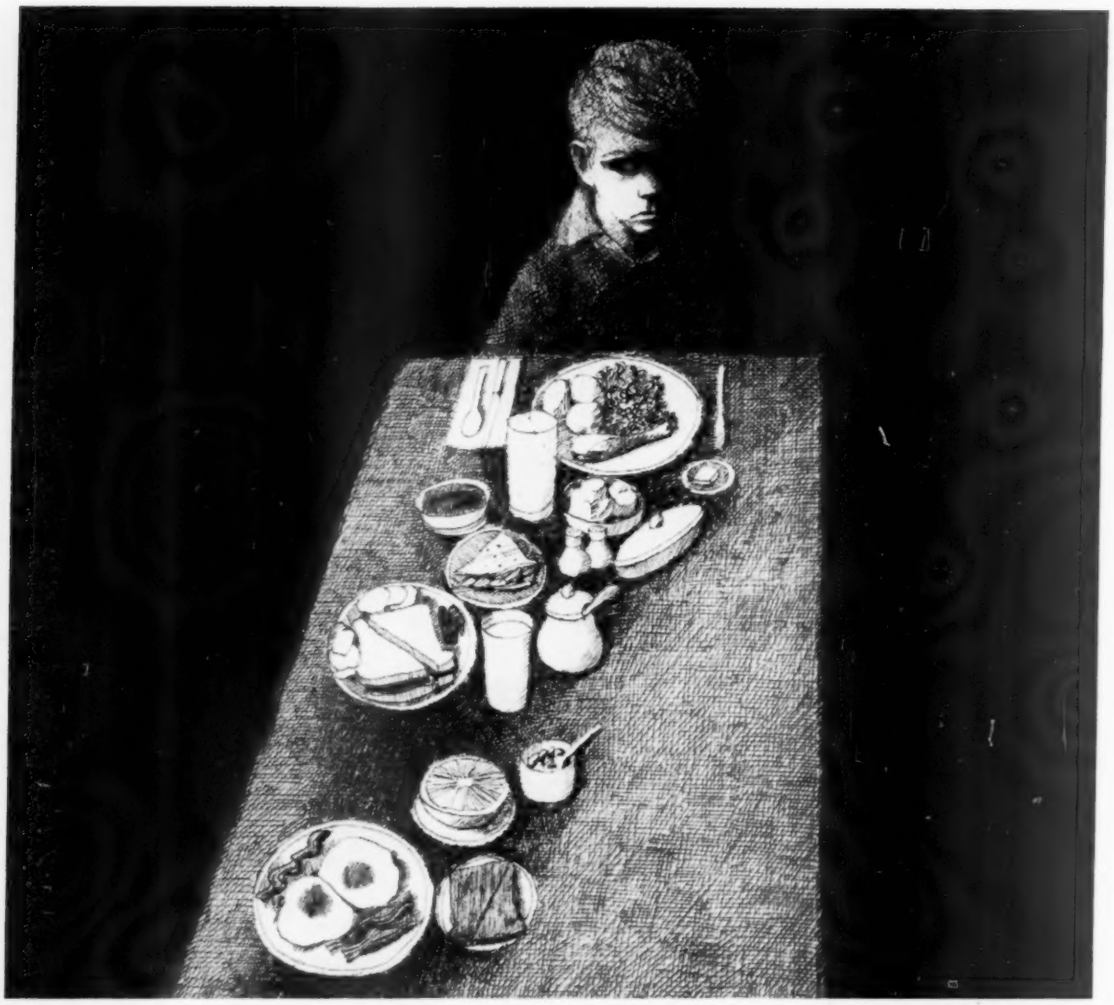
diagnosis: a wrought-up patient with a functional gastro-intestinal disorder compounded by inadequate digestion. **treatment:** reassurance first, then medication to relieve the gastric symptoms, calm the emotions, and enhance the digestive process. **prescription:** new Donnazyme—providing the multiple actions of widely accepted Donnatal® and Entozyme®—two tablets t.i.d., or as necessary.

Each Donnazyme tablet contains
—In the gastric-soluble outer layer: Hyoscyamine sulfate, 0.0518 mg.; Atropine sulfate, 0.0097 mg.; Hyoscine hydrobromide, 0.0033 mg.; Phenobarbital (1/8 gr.), 8.1 mg.; and Pepsin, N. F., 150 mg. **In the enteric-coated core:** Pancreatin, N. F., 300 mg., and Bile salts, 150 mg.

ANTISPASMODIC - SEDATIVE - DIGESTANT

DONNAZYME®

A. H. ROBINS COMPANY, INCORPORATED • RICHMOND 20, VIRGINIA



Vesprin

Squibb Triflupromazine Hydrochloride

controls anxiety and tension in everyday practice

Doctor...
I can reason
with him on
other problems...
but not when
it comes to
eating

In pediatric practice, as well as in general medical practice, many children with behavior problems can benefit from the administration of VESPRIN, a full range tranquilizer with minimal toxicity and side effects. Equally important to the physician is the fact that VESPRIN does not produce somnolence while retaining high potency as a tranquilizer – even in low dosage.

Consult Vesprin package insert or PDR for indications, dosage and directions for use. Supply: for oral use—tablets containing 10, 25 and 50 mg. **SQUIBB** *Squibb Quality – the Priceless Ingredient*

"VESPRIN"® IS A SQUIBB TRADEMARK

Preludin®

brand of phenmetrazine hydrochloride

whether obesity is simple or complicated

Through the potent appetite-suppressant action of Preludin, the success of anti-obesity treatment becomes more assured—adherence to diet becomes easier—discomfort from side reactions is unlikely.

In Simple Obesity
Preludin produces 2 to 5 times the weight loss achievable by dietary instruction alone.[1,2]

In Pregnancy
Weight gain is kept within bounds, without danger to either mother or fetus.[3]

In Diabetes
Insulin requirements are not increased; they may even decrease as weight is lost.[4]

In Hypertension
Preludin is well tolerated and blood pressure may even fall as weight is reduced.[1]

Preludin® Endurets™ (brand of phenmetrazine hydrochloride), prolonged-action tablets of 75 mg. for once daily administration; and scored, square, pink tablets of 25 mg. for b.i.d. or t.i.d. administration.

Under license from C. H. Boehringer Sohn, Ingelheim

References:
(1) Barnes, R. H.: J.A.M.A. *166*:898, 1958. (2) Ressler, C.: J.A.M.A. *165*:135, 1957. (3) Birnberg, C. H., and Abitbol, M. M.: Obst. & Gynec. *11*:463, 1958. (4) Robillard, R.: Canad. M.A.J. *76*:938, 1957.

Geigy, Ardsley, New York

Geigy

PR5-60

first of a new class of therapeutic agents for superior, safer, faster control of common emotional disturbances

in a class by itself— chemically

Not a manipulated molecule, the structure of this compound resembles that of no other drug. Librium is a product of truly original Roche research.

in a class by itself— pharmacologically

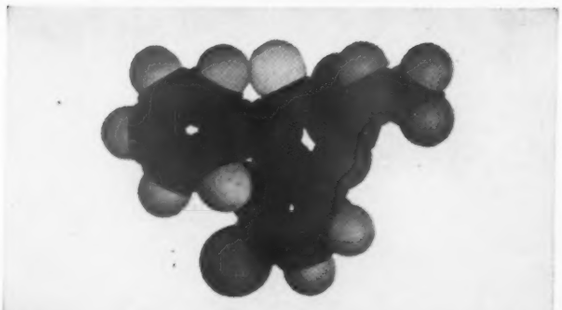
Librium exhibits an unprecedented "taming" action in animals. It is the first compound in which the specific antiaggressive component is separated from a generalized depressant effect on locomotor activity and reflex patterns. While Librium has tranquilizing properties comparable with those of chlorpromazine and reserpine, it lacks the autonomic blocking effects of these compounds and does not produce extrapyramidal side effects. Librium has none of the hypnotic effects of the barbiturates.

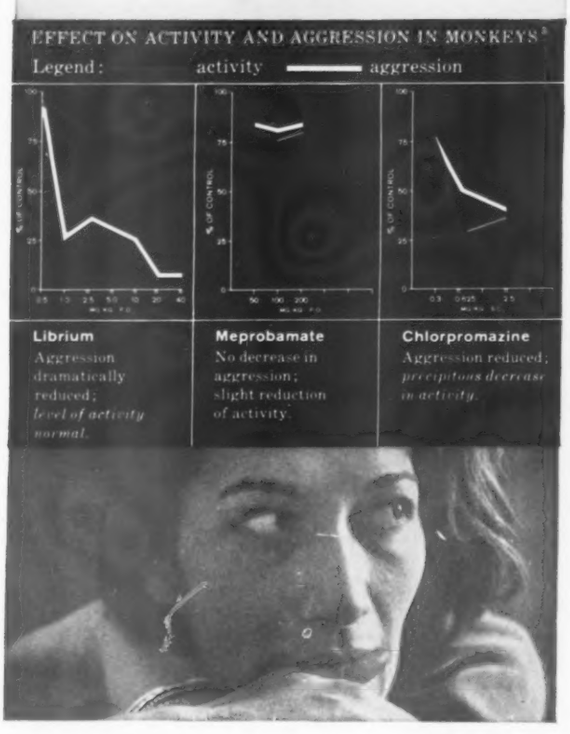
in a class by itself— clinically

The therapeutic range of Librium "envelops and extends well beyond that of meprobamate and into certain indications for which the phenothiazines are prescribed."[1] More than replacement therapy, Librium is quantitatively and qualitatively superior to "tranquilizers" and "equanimity-producing drugs." Librium is distinguished by an unusually rapid onset of action and a high degree of safety.

Published reports on Librium: 1. G. A. Constant, *Dis. Nerv. System*, 21:(Suppl.), 37, 1960. 2. T. H. Harris, *ibid.*, p. 3. 3. L. O. Randall, *ibid.*, p. 7. 4. H. A. Bowes, *ibid.*, p. 20. 5. J. M. Tobin, I. F. Bird and D. E. Boyle, *ibid.*, p. 11. 6. J. Kinross-Wright, I. M. Cohen and J. A. Knight, *ibid.*, p. 23. 7. H. H. Farb, *ibid.*, p. 27. 8. C. Breitner, *ibid.*, p. 31. 9. I. M. Cohen, *ibid.*, p. 35. 10. L. J. Thomas, *ibid.*, p. 40. 11. R. C. V. Robinson, *ibid.*, p. 43. 12. S. C. Kaim and I. N. Rosenstein, *ibid.*, p. 46. 13. H. E. Ticktin and J. D. Schultz, *ibid.*, p. 49. 14. J. N. Sussex, *ibid.*, p. 53. 15. I. N. Rosenstein, *ibid.*, p. 57. 16. I. N. Rosenstein and C. Silverblatt, to be published.

NEW LIBRIUM




EFFECT ON ACTIVITY AND AGGRESSION IN MONKEYS
Legend: activity aggression
% OF CONTROL
Librium
Aggression dramatically reduced; level of activity normal.
Meprobamate
No decrease in aggression; slight reduction of activity.
Chlorpromazine
Aggression reduced; precipitous decrease in activity.

to free the patient

from anxiety and tension, whether presenting symptomatology or associated with organic or functional disorders.

to free the therapy

from the drawbacks of previous agents.

to free the physician

from the frustrations of prolonged, inconclusive treatment and to render the patient more amenable to therapy.

uses of Librium

in the office patient, troubled by anxiety and tension, and by the irritability, fatigue and nervous insomnia associated with tension states

in the office patient, where you suspect anxiety and tension as contributing or causative factors of organic or functional disorders

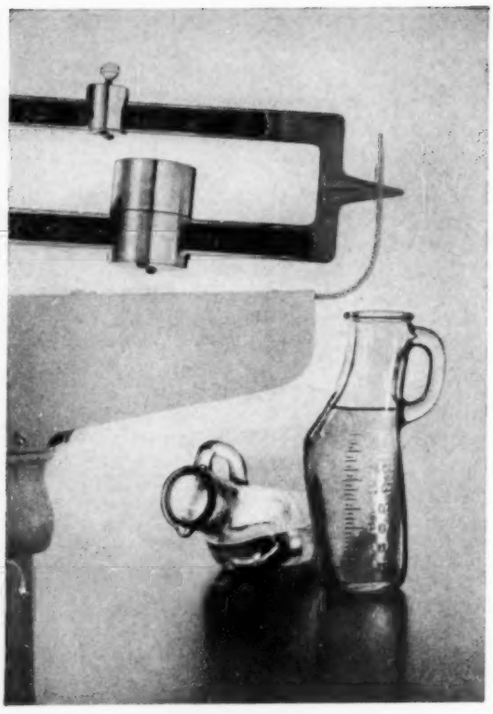
in more severely disturbed patients, including cases of agitated and reactive depression, fears, phobias, obsessions and compulsions

LIBRIUM™ Hydrochloride — 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine 4-oxide hydrochloride

ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc.
Nutley 10, N. J.

Supplied: 10-mg, green-and-black capsules. Bottles of 50 and 500.
For complete information regarding dosage and precautions, please consult product literature.

6 important reasons why physicians choose Esidrix for *congestive heart failure, toxemia & edema of pregnancy, premenstrual edema, steroid-induced edema, edema of obesity*



1. Esidrix is one of the most effective oral diuretics known... 10 to 15 times more active than chlorothiazide.
2. Esidrix markedly increases sodium and chloride excretion, usually with minimal effect on excretion of potassium and bicarbonate.
3. Certain patients unresponsive to mercurials and chlorothiazide respond readily to Esidrix.
4. Esidrix alone can be used to reduce blood pressure. It also potentiates the action of such other antihypertensive agents as Singoserp, Serpasil, Apresoline, and ganglionic blockers.
5. Patients transferring from chlorothiazide to Esidrix frequently experience additional loss of retained body fluid and/or lower blood pressure levels.
6. In most cases, Esidrix permits moderation in severe sodium restriction, makes meals more palatable.

Esidrix®

(hydrochlorothiazide CIBA)

An improved analog of chlorothiazide, a product of CIBA research

SUPPLIED: Esidrix *Tablets*, 25 mg. (pink, scored) and 50 mg. (yellow, scored).

Complete information available on request.

CIBA
SUMMIT, NEW JERSEY

SINGOSERP® (syrosingopine CIBA)
SERPASIL® (reserpine CIBA)
APRESOLINE® hydrochloride (hydralazine hydrochloride CIBA) 2/2800HK

everything under control— including the temperature

with **Tylenol®**

FIRST LIQUID PEDIATRIC ANTIPYRETIC/ ANALGESIC

"... a safe and effective agent in the control of fever at any age in contrast to other agents which are of known toxicity ..."

"... this drug [acetaminophen] has been used extensively by our group without ill effect."[1]

Tylenol® *Acetaminophen*

relieves pain and brings fever under control quickly, safely ... is well liked by children.

In colds, "flu," sore throats – ℞ Tylenol for prompt relief of fever, headache, general discomfort.

2 dosage forms:

TYLENOL ELIXIR
120 mg. (2 gr.) per 5 cc.; 4 and 12 oz. bottles.

TYLENOL DROPS
60 mg. (1 gr.) per 0.6 cc.; 15 cc. bottles with calibrated droppers.

McNEIL

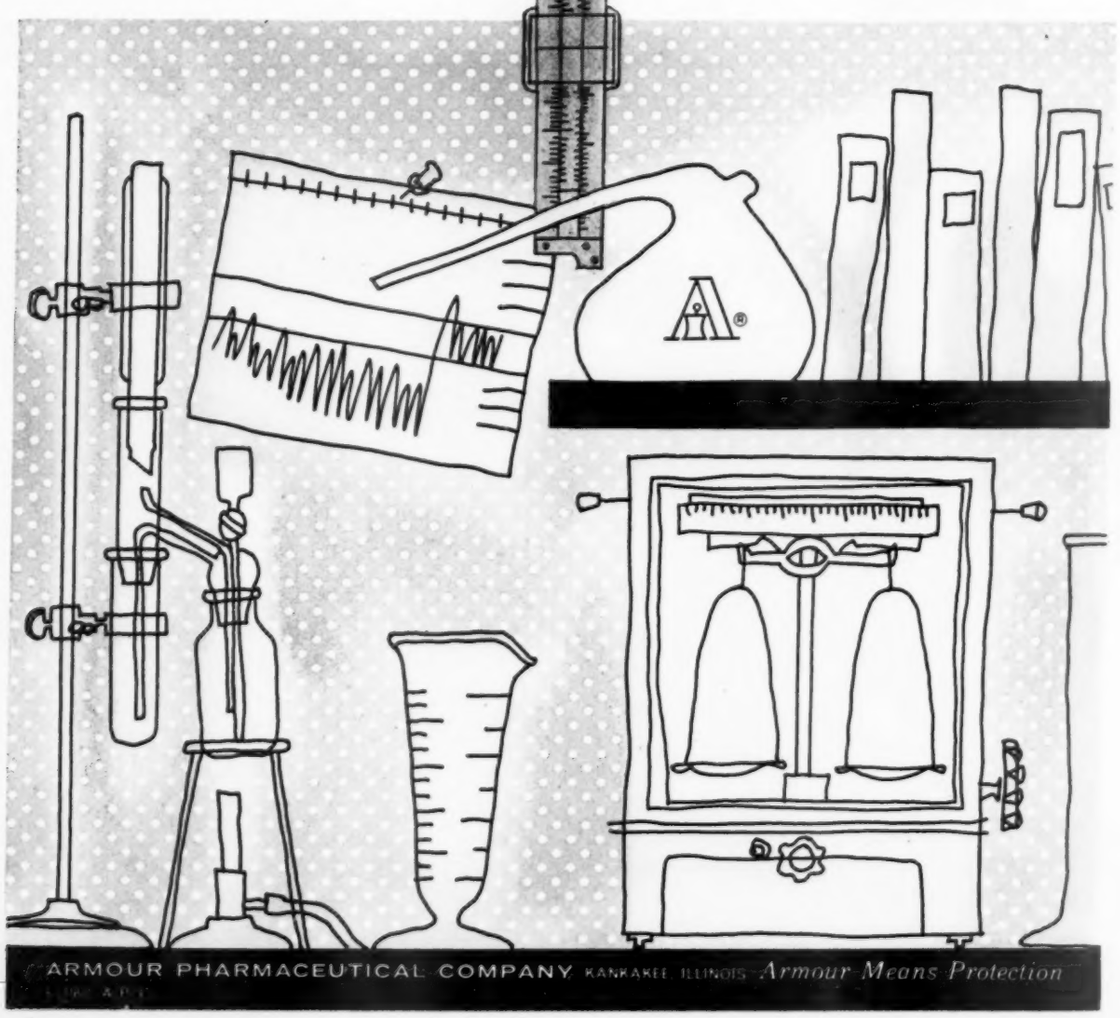
McNEIL LABORATORIES, INC., PHILADELPHIA 32, PA.

1. Mintz, A. A.: Management of the Febrile Child, J. Ky. Acad. Gen. Pract. 5:26 (Jan.) 1959.

ALWAYS SPECIFY ARMOUR THYROID

ARMOUR THYROID for over half a century has been more widely prescribed...more widely dispensed than any other thyroid product. Pioneer in thyroid standardization, Armour's rich background of experience assures you of unsurpassed quality, uniform potency and consistent therapeutic effects.

BY ANY MEASUREMENT THE THYROID OF CHOICE



ARMOUR PHARMACEUTICAL COMPANY KANKAKEE, ILLINOIS *Armour Means Protection*

LOST & FOUND

OTHER LOST & FOUND—PAGE 1

LOST, lady's diamond engagement ring. Center diamond 90 points and 2 side diamonds. Initials "I.J.E. to J.J.S., 4/6/58." Vicinity 32d & Chestnut sts., on Oct. 26. Liberal reward. W. E. Hunter & Sons, WA 5-7611.

LOST—Oct 30th. Vic Bellevue Stratford Hotel & 30th Railroad Station. Gold clip-pin. Scroll design, rubies & diamonds. Liberal reward. Call collect Roanoke, Virginia. Dickens 5-9303.

LOST—Mon eve, U of P vic. Black male puppy, Kerry Blue Terrier. No collar. Left ear pinned down. Lg rew. EV 6-7094 or EV 6-5502.

LOST: 9,000 tons of human fat, by patients whose doctors prescribed Obedrin.

LOST—Penna Railroad Pass in wallet. Reward. Return to Mr. W. H. Hall, 1804 N 21st st or CE 2-1012.

LOST—Brown brief case cont Bibles, stop watch, etc, vic 15th & Girard. Reward. $10. Howe 10 6-7365.

LOST—Ladies' gold Omega watch, blue crystal, vic Wanamaker's. Oct 31. Reward. HAzel 6-4300 (N J).

LOST Nov 3. Beaded black handbag. Gift of a concrete mixer. Rew. Ph 7-6060. Vic Loesser Jr High.

LOST.—Star sapphire ring, 10/24, P M, vic Terrace theater or center Philc, reward. WA 7-6717.

LOST—Black pocketbook, vic Lewis's Broad St. Reward. IC 5-5750, Ext 2.

LOST. Green diamond watch on 14th st bet Spruce & Pine, kev. GR 6-4716

LOST. Bankbook # Girard

LOST

Over 300,000,000 tablets of Obedrin have been administered since its introduction . . . enabling overweight patients to lose approximately 18,000,000 pounds of excess fat. (Studies show an average weight loss of 6 lbs. per 100 tablets. *)

!!

Obedrin and the 60-10-70 Basic Plan provide the three essentials of weight reduction:

1 Supervision by the physician

2 A balanced eating plan

3 Supportive medication

Obedrin curbs unhealthy food craving, enabling the patient to establish correct eating habits . . . first, to lose excess pounds and then, more important, to maintain optimum weight.

Each capsule or tablet provides:	
	Semoxydrine® HCl (methamphetamine HCl), 5 mg., for its anorexigenic and mood-lifting effects
	Pentobarbital, 20 mg., to guard against excitation
	Thiamine Mononitrate, 0.5 mg., Riboflavin, 1 mg., and Nicotinic Acid (Niacin), 5 mg., to supplement the diet
	Ascorbic Acid, 100 mg., to help mobilize tissue fluids

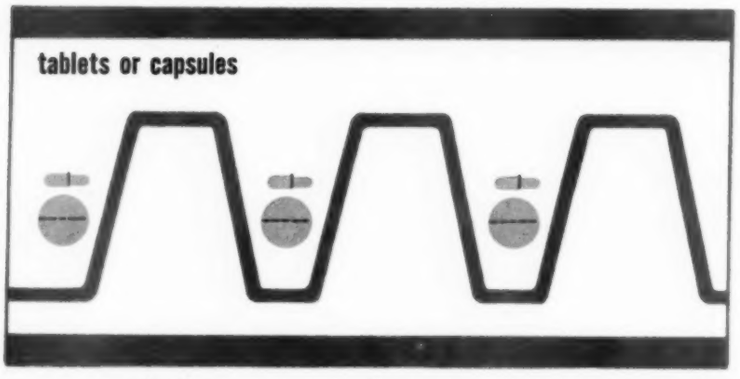
* Elsfelder, Henry W.: Treatment of Obesity. The Role of the Doctor, Drug and Diet in Weight Loss, Am. Pract. and Dig. of Treat., 5:778 (Oct., 1954). Sherman, Robert J.: Weight Reduction, Med. Times, 82:107 (Feb., 1954).

Obedrin®

and the 60-10-70 Basic Plan provide an effective weight-control regimen

Bristol, Tennessee • New York • Kansas City • San Francisco THE S. E. MASSENGILL COMPANY

A FLEXIBLE DOSAGE FORM PROVIDES DEPENDABLE CONTROL OF APPETITE



The Obedrin formula permits a flexible dosage schedule to depress the appetite at peak hunger periods. The physician can adjust dosage to fit each patient's need.

ADVANTAGES OF OBEDRIN—

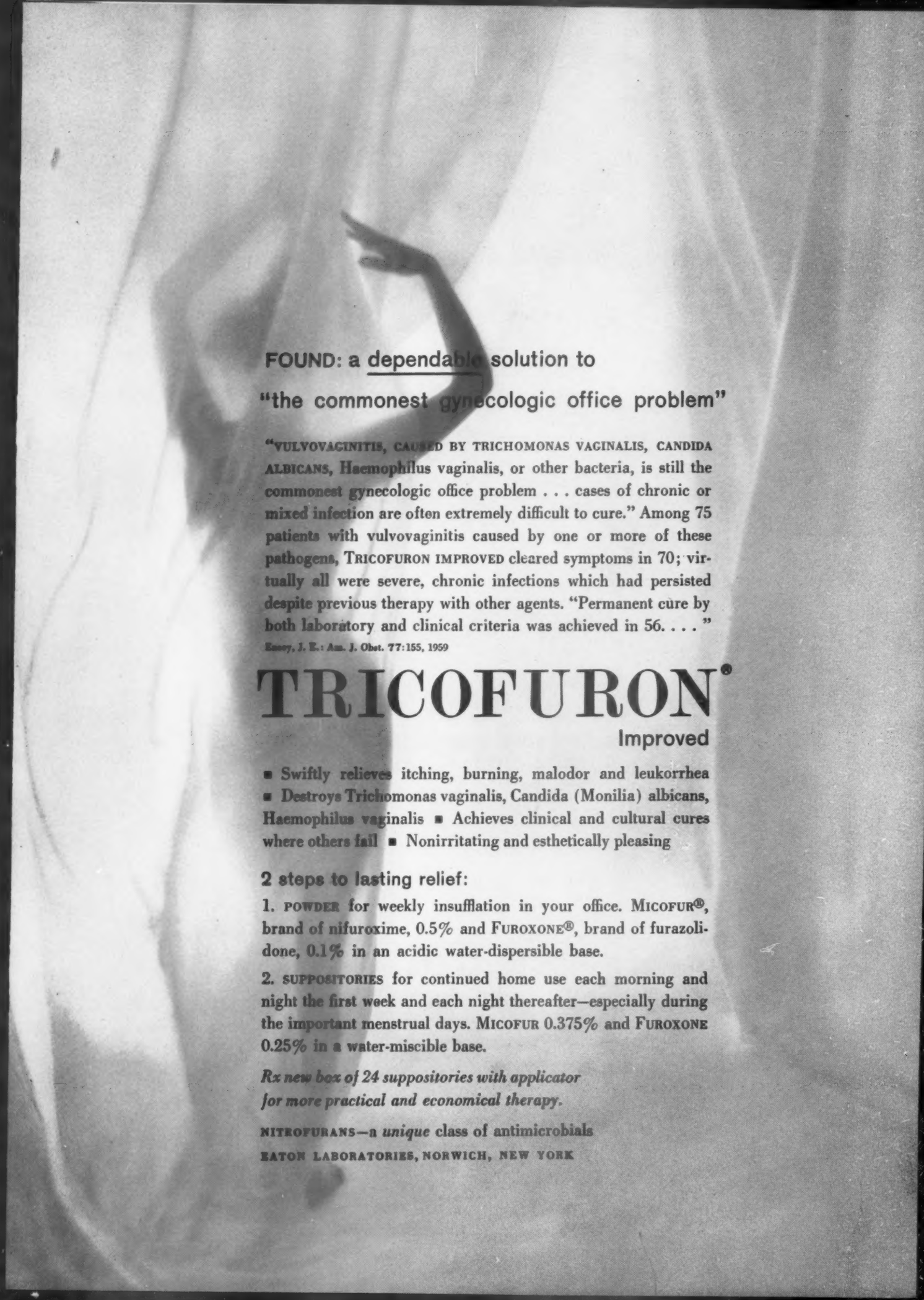
A dependable anorexigenic agent!
A flexible dosage form!
Minimal central nervous stimulation!
Vitamins to supplement the diet!
No hazards of impaction!!!

Write for 60-10-70 menus, weight charts, and Obedrin samples. Used with the 60-10-70 Basic Plan, Obedrin offers an ideal weight-control regimen for the overweight patient.

Obedrin®

and the 60-10-70 Basic Plan

Bristol, Tennessee • New York • Kansas City • San Francisco THE S. E. MASSENGILL COMPANY



FOUND: a dependable solution to "the commonest gynecologic office problem"

"VULVOVAGINITIS, CAUSED BY TRICHOMONAS VAGINALIS, CANDIDA ALBICANS, Haemophilus vaginalis, or other bacteria, is still the commonest gynecologic office problem . . . cases of chronic or mixed infection are often extremely difficult to cure." Among 75 patients with vulvovaginitis caused by one or more of these pathogens, TRICOFURON IMPROVED cleared symptoms in 70; virtually all were severe, chronic infections which had persisted despite previous therapy with other agents. "Permanent cure by both laboratory and clinical criteria was achieved in 56. . . . "

Essey, J. E.: Am. J. Obst. 77:155, 1959

TRICOFURON®

Improved

■ Swiftly relieves itching, burning, malodor and leukorrhea ■ Destroys Trichomonas vaginalis, Candida (Monilia) albicans, Haemophilus vaginalis ■ Achieves clinical and cultural cures where others fail ■ Nonirritating and esthetically pleasing

2 steps to lasting relief:

1. POWDER for weekly insufflation in your office. MICOFUR®, brand of nifuroxime, 0.5% and FUROXONE®, brand of furazolidone, 0.1% in an acidic water-dispersible base.

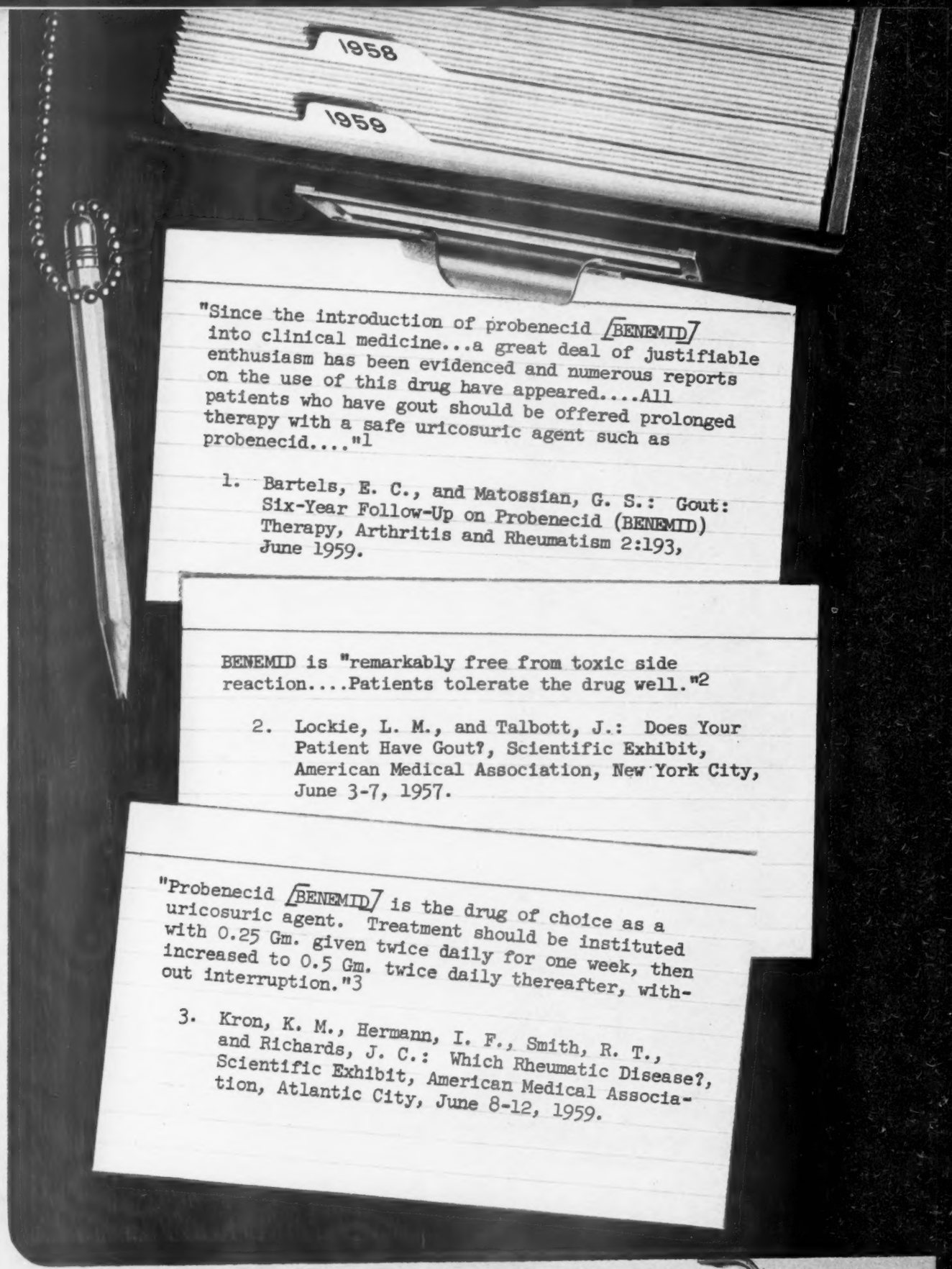
2. SUPPOSITORIES for continued home use each morning and night the first week and each night thereafter—especially during the important menstrual days. MICOFUR 0.375% and FUROXONE 0.25% in a water-miscible base.

Rx new box of 24 suppositories with applicator for more practical and economical therapy.

NITROFURANS—a *unique* class of antimicrobials

EATON LABORATORIES, NORWICH, NEW YORK

1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
SINCE 1950...TEN YEARS OF GROWING CONFIDENCE
IN THE EFFECTIVENESS AND SAFETY OF
BENEMID®
PROBENECID
IN GOUT



"Since the introduction of probenecid [BENEMID] into clinical medicine...a great deal of justifiable enthusiasm has been evidenced and numerous reports on the use of this drug have appeared....All patients who have gout should be offered prolonged therapy with a safe uricosuric agent such as probenecid...."1

1. Bartels, E. C., and Matossian, G. S.: Gout: Six-Year Follow-Up on Probenecid (BENEMID) Therapy, Arthritis and Rheumatism 2:193, June 1959.

BENEMID is "remarkably free from toxic side reaction....Patients tolerate the drug well."2

2. Lockie, L. M., and Talbott, J.: Does Your Patient Have Gout?, Scientific Exhibit, American Medical Association, New York City, June 3-7, 1957.

"Probenecid [BENEMID] is the drug of choice as a uricosuric agent. Treatment should be instituted with 0.25 Gm. given twice daily for one week, then increased to 0.5 Gm. twice daily thereafter, without interruption."3

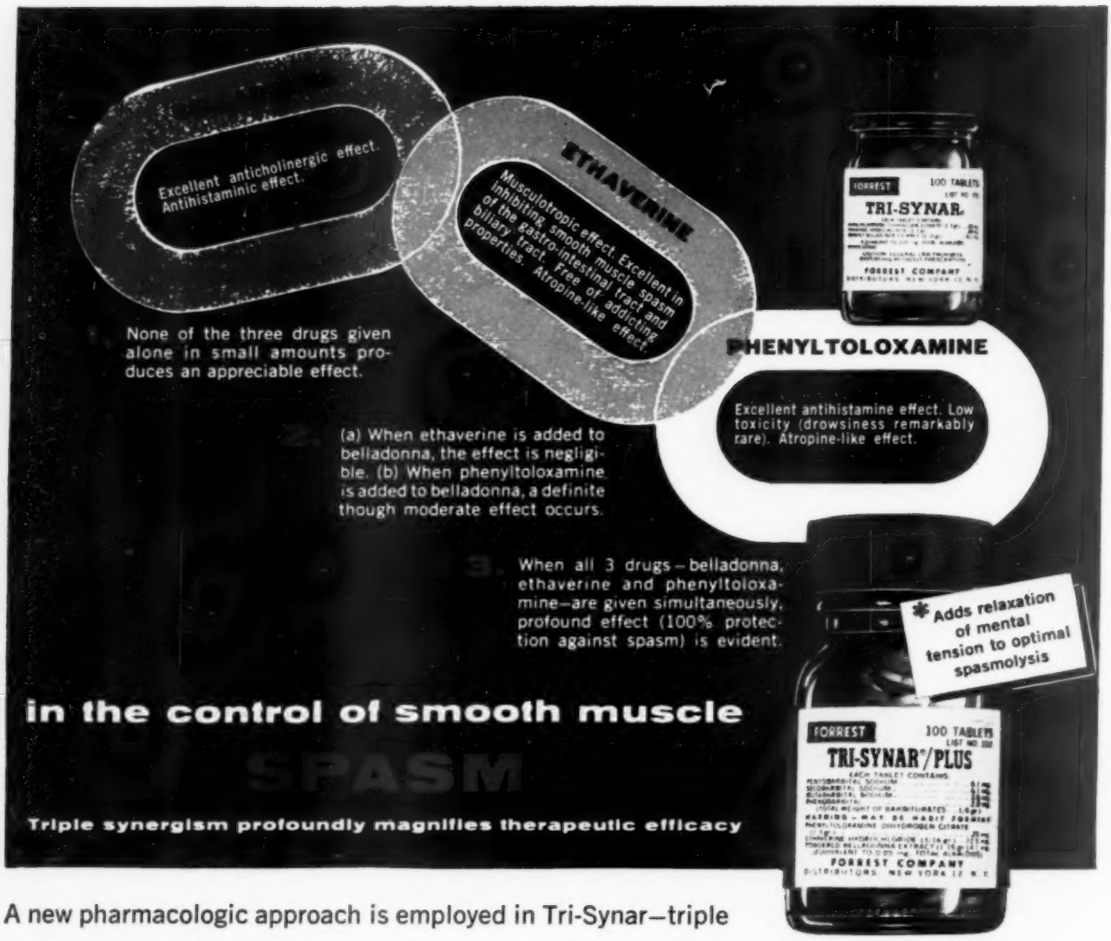
3. Kron, K. M., Hermann, I. F., Smith, R. T., and Richards, J. C.: Which Rheumatic Disease?, Scientific Exhibit, American Medical Association, Atlantic City, June 8-12, 1959.

Supply: 0.5 Gm. tablets, bottles of 100 and 1000.

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.

MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

BENEMID IS A TRADEMARK OF MERCK & CO., INC.


Excellent anticholinergic effect. Antihistaminic effect.
None of the three drugs given alone in small amounts produces an appreciable effect.
ETHAVERINE
Musculotropic effect. Excellent in inhibiting smooth muscle spasm of the gastro-intestinal tract and biliary tract. Free of addicting properties. Atropine-like effect.
(a) When ethaverine is added to belladonna, the effect is negligible. (b) When phenyltoloxamine is added to belladonna, a definite though moderate effect occurs.
PHENYLTOLOXAMINE
Excellent antihistamine effect. Low toxicity (drowsiness remarkably rare). Atropine-like effect.
When all 3 drugs – belladonna, ethaverine and phenyltoloxamine – are given simultaneously, profound effect (100% protection against spasm) is evident.
FORREST
100 TABLETS
TRI-SYNAR
FORREST COMPANY
* Adds relaxation of mental tension to optimal spasmolysis
in the control of smooth muscle
SPASM
Triple synergism profoundly magnifies therapeutic efficacy
FORREST
100 TABLETS
TRI-SYNAR*/PLUS
FORREST COMPANY

A new pharmacologic approach is employed in Tri-Synar—triple synergism—which makes possible for the first time, powerful sedation with small doses of belladonna. Tri-Synar attacks smooth muscle spasm 3 ways...musculotropic, anticholinergic and antihistaminic. The protective action of the constituents of Tri-Synar against a standardized experimental smooth muscle spasm was studied in successive steps, starting with the use of the single drugs (1), followed by the use of the drugs in pairs (2), and finally the use of all three drugs (3).

INDICATIONS: Spastic and functional conditions of the gastrointestinal tract (including spastic colitis, epigastric distress, adjunctive therapy in peptic ulcer, spastic constipation), biliary syndrome (dyskinesia, choleystitis), primary dysmenorrhea, vomiting of pregnancy, dysuria and mild ureteral spasms. **CONTRAINDICATIONS:** Glaucoma, lower urinary tract obstruction, or pyloric obstruction due to stenosis or scarring.

Each tablet contains:

	TRI-SYNAR	TRI-SYNAR PLUS
Powdered Extract of Belladonna*	4.1 mg.	4.1 mg.
Phenyltoloxamine Dihydrogen Citrate	20.0 mg.	20.0 mg.
Ethaverine Hydrochloride	20.0 mg.	12.5 mg.
Secobarbital Sodium		6.075 mg.
Pentobarbital Sodium		6.075 mg.
Butabarbital Sodium		2.025 mg.
Phenobarbital		2.025 mg.

*The belladonna is equivalent to 2.5 minims of tincture of belladonna, U.S.P.

DOSAGE: 1 tablet t.i.d. or q.i.d.; in the more severe cases, 2 tablets t.i.d.

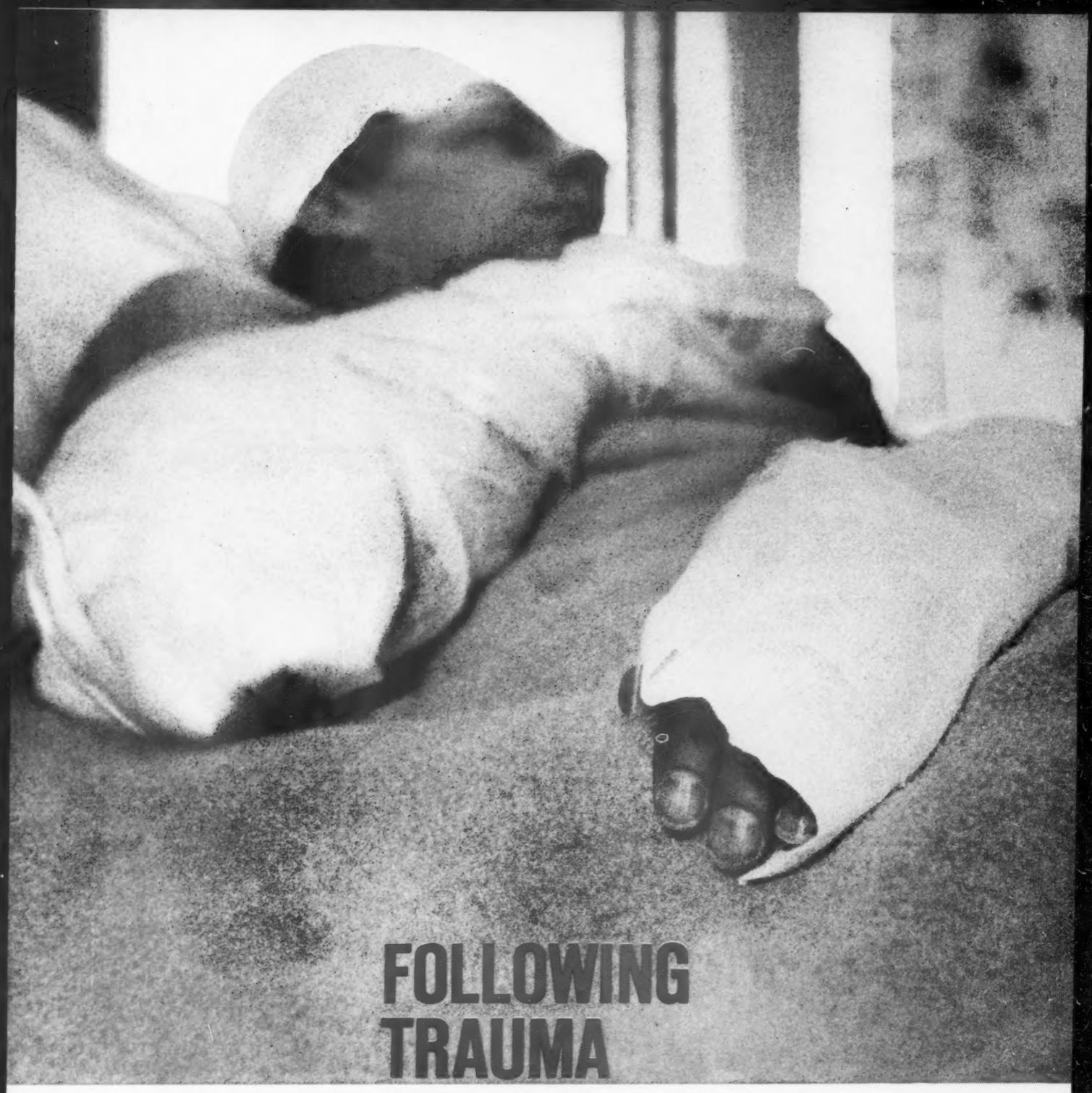
SUPPLIED: Bottles of 100 tablets

TRI-SYNAR and TRI-SYNAR / PLUS*

See page 676 of your Physicians' Desk Reference—

CLINICAL SUPPLY AND LITERATURE AVAILABLE ON REQUEST

FORREST COMPANY • 93 CROSBY STREET, NEW YORK 12, N.Y.



FOLLOWING TRAUMA

nutritional therapy in the "therapeutic" jar

STRESSCAPS helps meet increased metabolic requirements in burns, fractures and wounds. Abnormal levels of water-soluble vitamins are suddenly required with other nutritional factors—just as the stress reaction induces severe depletion[1,2] and alters metabolism.[3] High potency supplements must be administered[1,2] as provided by STRESSCAPS, to support rapid recovery and prevent general complications of metabolic failure. Of "therapeutic" importance to the out-patient, the attractive STRESSCAPS jar is a convenient reminder of daily dosage... insuring adequate intake over the therapeutic course.

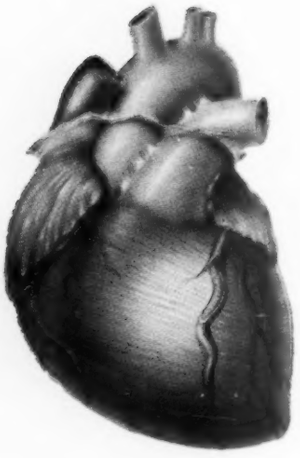
Each capsule contains: Thiamine Mononitrate (B_1) 10 mg., Riboflavin (B_2) 10 mg., Niacinamide 100 mg., Ascorbic Acid (C) 300 mg., Pyridoxine HCl (B_6) 2 mg., Vitamin B_{12} 4 mcgm., Folic Acid 1.5 mg., Calcium Pantothenate 20 mg., Vitamin K (Menadione) 2 mg. Average dose: 1-2 capsules daily.

1. Richardson, M. E.: J. Am. Osteop. A. 57:562 (May) 1958. 2. Mason, M. L.: Northwest Med. 57:1439 (Nov.) 1958. 3. Coleman, S. S.: Am. J. Surg. 97:43 (Jan.) 1959.

LEDERLE LABORATORIES, a Division of AMERICAN CYANAMID COMPANY, Pearl River, New York

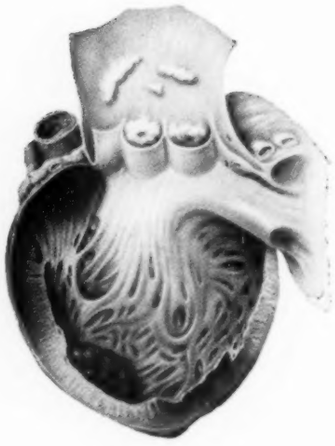
STRESSCAPS®

Stress Formula Vitamins Lederle



IN ANGINA PECTORIS AND CORONARY INSUFFICIENCY

. . . the treatment must go further than vasodilation alone. It should also control the patient's ever-present anxiety about his condition, since anxiety itself may bring on further attacks.



AFTER MYOCARDIAL INFARCTION

. . . it is frequently not enough to boost blood flow through arterial offshoots and establish new circulation. The disabling fear and anxiety that invariably accompany the condition must be reduced, or the patient may become a chronic invalid.

Protects your coronary patient better than vasodilation alone

Unless the coronary patient's ever-present anxiety about his condition can be controlled, it can easily induce an anginal attack or, in cases of myocardial infarction, considerably delay recovery.

This is why Miltrate gives better protection for the heart than vasodilation alone in coronary insufficiency, angina pectoris and postmyocardial infarction. Miltrate contains not only PETN (pentaerythritol tetranitrate), acknowledged as basic therapy for long-acting vasodilation. What is more important – Miltrate provides Miltown, a tranquilizer of *proven* effectiveness in relieving anxieties, fear and day-to-day tension in over 600 clinical studies.

Thus, your patient's cardiac reserve is protected against his fear and concern about his condition...and his operative arteries are dilated to enhance myocardial blood supply.

Supplied: Bottles of 50 tablets. Each tablet contains 200 mg. Miltown and 10 mg. pentaerythritol tetranitrate.
Dosage: 1 or 2 tablets q.i.d. *before meals* and at bedtime, according to individual requirements.

REFERENCES

1. Ellis, L. B. *et al.:* Circulation *17*:945, May 1958. **2.** Friedlander, H. S.: Am. J. Cardiol. *1*:395, Mar. 1958. **3.** Riseman, J. E. F.: New England J. Med. *261*:1017, Nov. 12, 1959. **4.** Russek, H. I. *et al.:* Circulation *12*:169, Aug. 1955. **5.** Russek, H. I.: Am. J. Cardiol. *3*:547, April 1959. **6.** Tortora, A. R.: Delaware M. J. *30*:298, Oct. 1958. **7.** Waldman, S. and Pelner, L.: Am. Pract. & Digest Treat. *8*:1075, July 1957.

Miltrate®

Miltown® (meprobamate) + PETN

CML-1388

WALLACE LABORATORIES / *New Brunswick, N. J.*

Butazolidin®

brand of phenylbutazone

in arthritis and allied disorders

proved by a decade of experience

Ten years of experience in countless cases—more than 1700 published reports—have now established the eminence of Butazolidin among the potent non-hormonal antiarthritic agents.

Repeatedly it has been demonstrated that Butazolidin:
Within 24 to 72 hours produces striking relief of pain.
Within 5 to 10 days affords a marked improvement in mobility and a significant subsidence of inflammation with reduction of swelling and absorption of effusion.

Even when administered over months or years Butazolidin does not provoke tolerance nor produce signs of hormonal imbalance.

Geigy

Butazolidin® (brand of phenylbutazone):
Red-coated tablets of 100 mg.
Butazolidin® Alka: Capsules containing Butazolidin® 100 mg.; dried aluminum hydroxide gel 100 mg.; magnesium trisilicate 150 mg.; homatropine methylbromide 1.25 mg.

Geigy, Ardsley, New York

Back again

with renewed joint pain and stiffness...discouraged, worried, dissatisfied. Her morale alone demands a new approach. But what?

This time...ATARAXOID®

prednisolone-hydroxyzine HCl

IN RHEUMATOID ARTHRITIS

Combines the established steroid, prednisolone (Sterane®) with tension-easing hydroxyzine HCl. When anxiety impedes clinical response, ATARAXOID offers superior control—often at lower steroid dosage in the case of certain rheumatic disorders—and without *unexpected* side effects.

also indicated in bronchial asthma and inflammatory/allergic dermatoses

ATARAXOID provides 10 mg. hydroxyzine HCl with various potencies of prednisolone per tablet: **ATARAXOID *5.0*** scored, green tablets, 5 mg. **ATARAXOID *2.5*** scored, blue tablets, 2.5 mg. **ATARAXOID *1.0*** scored, orchid tablets, 1 mg.

Professional Information Available on Request

PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. Brooklyn 6, New York Pfizer *Science for the world's well-being™*

Helps prevent vitamin-mineral deficiencies by providing comprehensive nutritional supplementation. Just one capsule daily supplies therapeutic doses of 9 important vitamins plus significant quantities of 11 essential minerals and trace elements.

Each MYADEC Capsule contains: VITAMINS: Vitamin B_{12} crystalline—5 mcg.; Vitamin B_2 (riboflavin)—10 mg.; Vitamin B_6 (pyridoxine hydrochloride)—2 mg.; Vitamin B_1 mononitrate—10 mg.; Nicotinamide (niacinamide)—100 mg.; Vitamin C (ascorbic acid)—150 mg.; Vitamin A—25,000 units; Vitamin D—1,000 units; Vitamin E (mixed tocopheryl acetates)—5 I.U.; MINERALS (as inorganic salts): Iodine—0.15 mg.; Manganese—1.0 mg.; Cobalt—0.1 mg.; Potassium—5.0 mg.; Molybdenum—0.2 mg.; Iron—15.0 mg.; Copper—1.0 mg.; Zinc—1.5 mg.; Magnesium—6.0 mg.; Calcium—105.0 mg.; Phosphorus—80.0 mg. Bottles of 30, 100, 250, and 1,000.

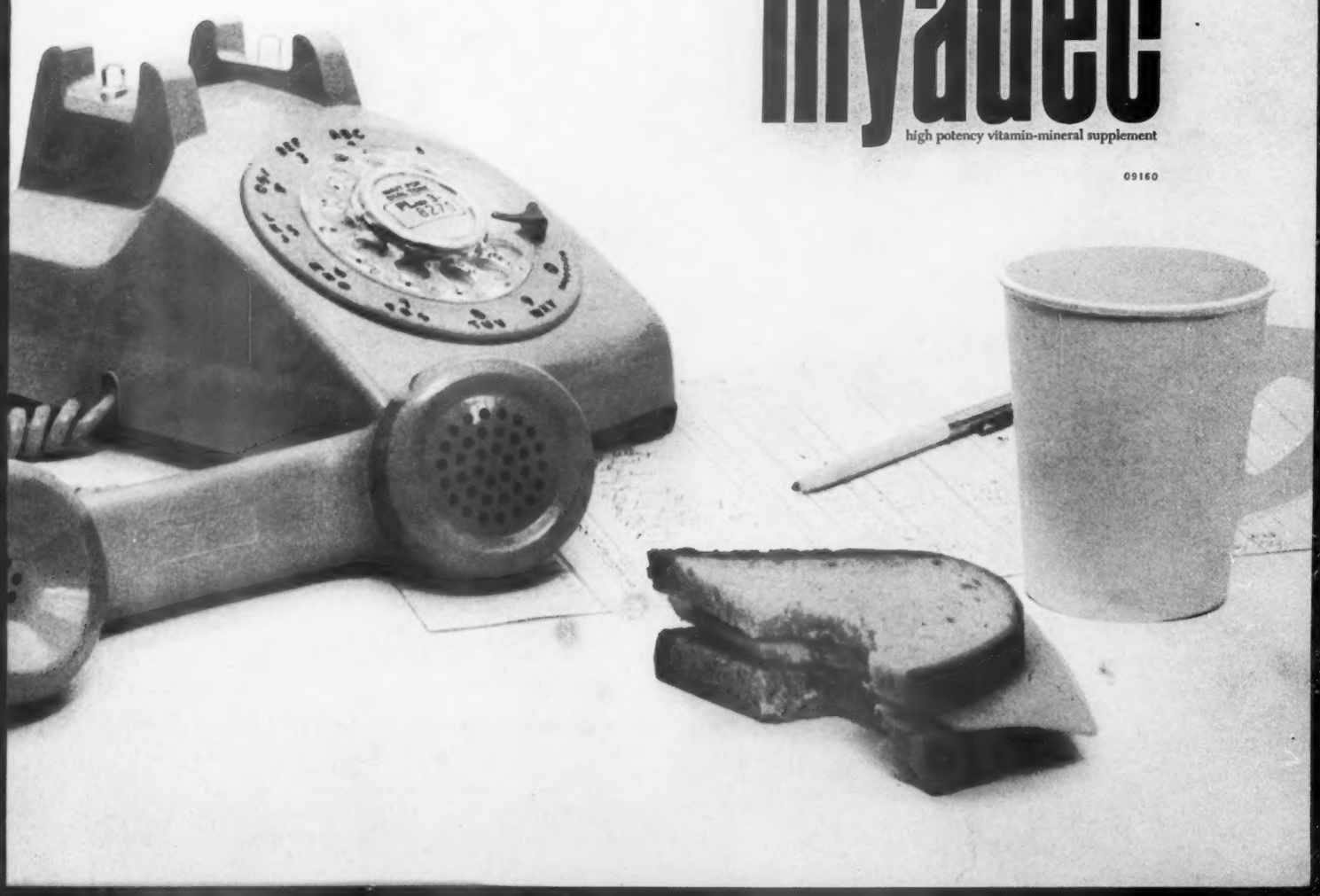
PARKE, DAVIS & COMPANY · DETROIT 32, MICHIGAN

when he sleeps through breakfast —and works through lunch...

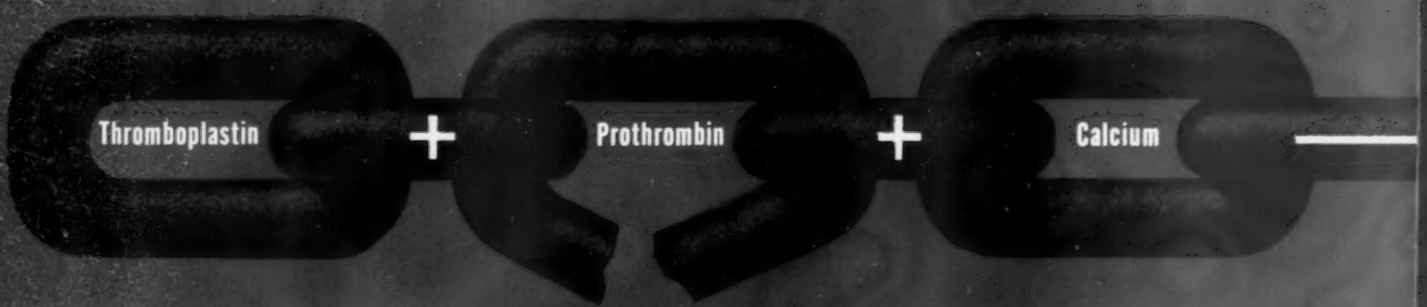
Myadec®

high potency vitamin-mineral supplement

09160



when the
weak link
in the
clotting chain is
anticoagulant-induced
hypoprothrombinemia



the preferred antidote is

Mep

For additional information, write Professional Services, Merck Sharp & Dohme, West Point, Pa.

"... has a more prompt, more potent and more prolonged effect than the vitamin K analogues.... Its reliability in treating undue hypoprothrombinemia from anticoagulant therapy is of particular importance. [Mephyton] can be depended on to reverse anticoagulant-induced hypoprothrombinemia to safe levels whether bleeding is only potential or actually has occurred."

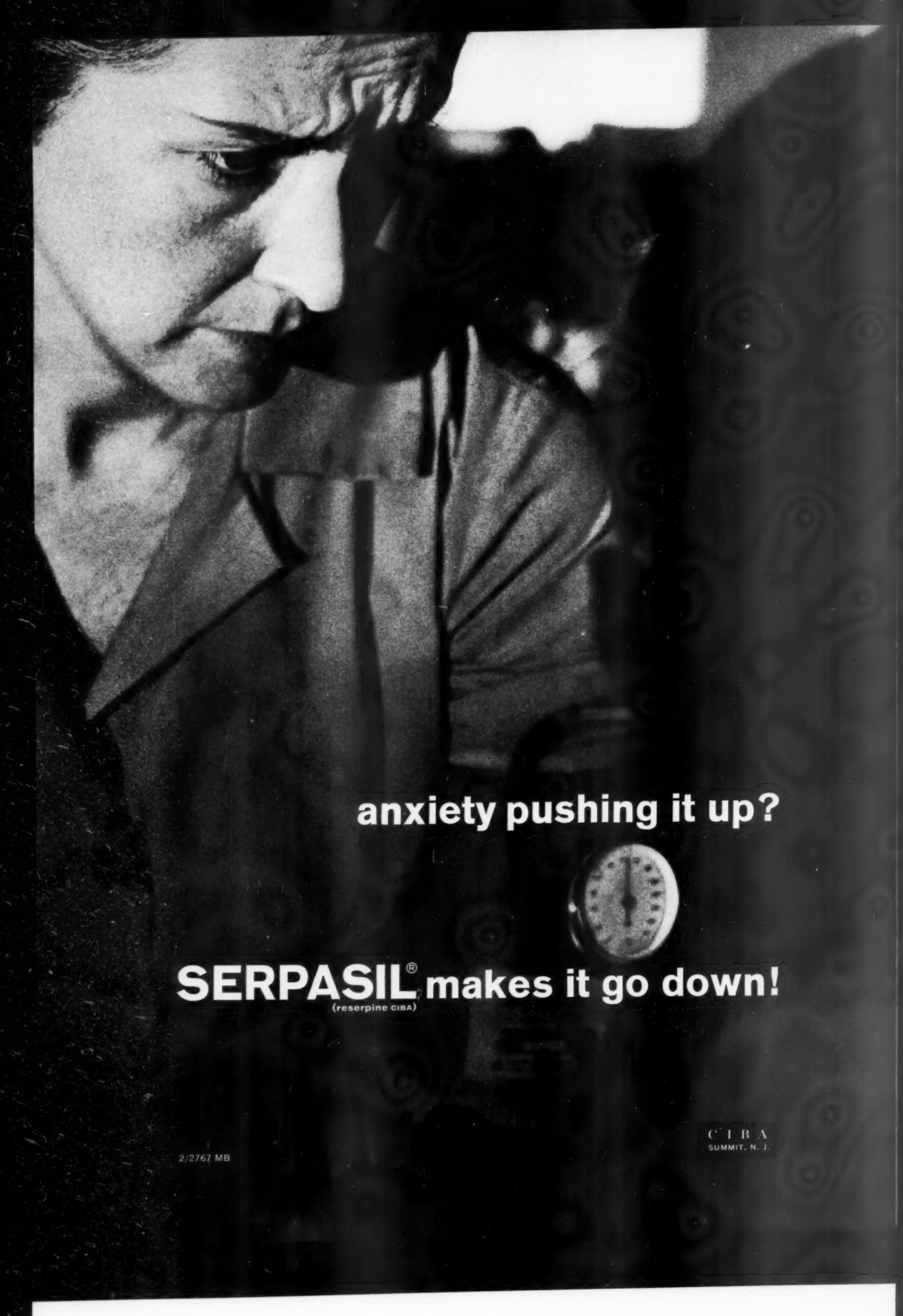
Council on Drugs: New and Nonofficial Drugs, Philadelphia, J. B. Lippincott Co., 1959, p. 661.

hyton®

vitamin K_1

Supply: Tablets 5 mg., bottles of 100. Emulsion, 1-cc. ampuls containing 10 mg. and 50 mg. per cc.; boxes of 6 ampuls.

MERCK SHARP & DOHME, DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.


anxiety pushing it up?
SERPASIL®
(reserpine CIBA)
makes it go down!
CIBA
SUMMIT, N. J.
2/2767 MB

Schering

yes! Polanil®

answers best the question of how to treat patients with allergic dermatoses.

When you prescribe POLANIL (composed of POLARAMINE®, today's lowest-dosage antihistamine, plus DERONIL®, today's lowest-dosage corticosteroid), you can control the discomfort of allergic dermatoses, hay fever and seasonal asthma. (Remember, too, POLARAMINE alone or in combination controls discomfort of seasonal and nonseasonal allergies; allergic complications of respiratory illness; drug and serum reactions.)

Because of its unique composition, POLANIL is particularly recommended for those dermatoses in which an antihistamine alone may not be fully effective, or for which full steroid therapy is not indicated. Pruritus responds favorably to POLANIL in almost all cases even when edema and erythema may persist.

POLANIL is effective in treating patients with resistant allergic dermatoses and seasonal asthma because the POLARAMINE component blocks the reception of histamine in precisely those areas where histamine is concentrated and where it provokes the most intense reaction: the skin, the upper gastrointestinal tract and the respiratory tree. The DERONIL component possesses an intensified anti-inflammatory activity with minimal effect on electrolyte and water balance.

Dosage: One or two tablets after meals and at bedtime. Dosage should be gradually reduced to lowest effective maintenance level or, if possible, discontinued.

Supply: Available in bottles of 50. Each tablet contains 0.25 mg. dexamethasone, 2 mg. dexchlorpheniramine maleate, and 75 mg. ascorbic acid. EN-1464

Cambridge

PULMONARY FUNCTION TESTER

This is a completely integrated, easy-to-use mobile instrument for the determination of residual volume or functional residual capacity by the closed circuit helium dilution technique. It provides data for the determination of the following values:

- **Functional Residual Capacity**
- **Vital Capacity**
- **Maximum Breathing Capacity**
- **Ventilation Equivalent**
- **Minute Ventilation**
- **Respiration Rate**
- **Tidal Volume**
- **Oxygen Uptake**

All the component sub-units which the user has hitherto been compelled to procure from various suppliers and to assemble for himself are built into this compact instrument. They include:

Cambridge Helium Analyzer with AC Power Unit

Collins 9-liter (or 13.5 liter) Respirometer with dead space occluder and Motor Blower

CO_2 Absorber and Resaturator

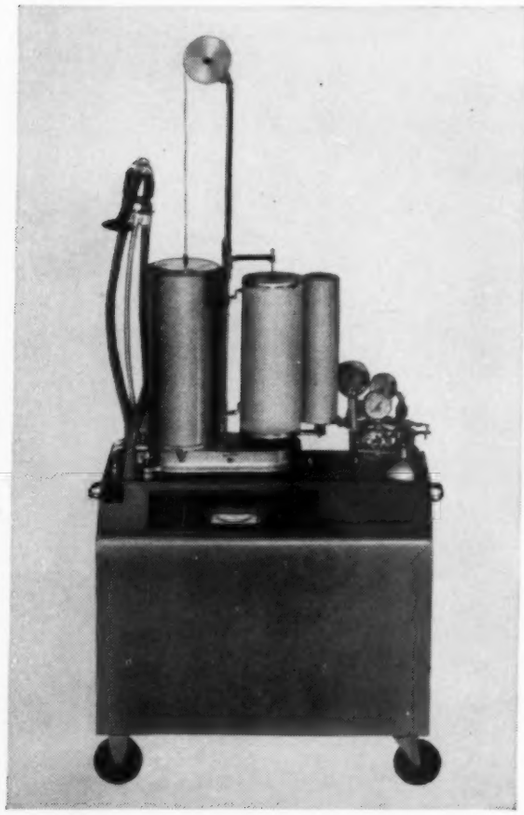
Oxygen and Helium Cylinders with yokes

Pressure Regulators and Oxygen Flowmeters

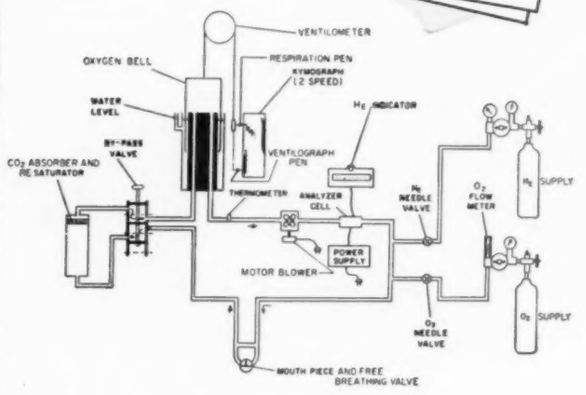
Piping and Valves

The Cambridge Pulmonary Function Tester is ideally suited for use in private offices, chest clinics, hospitals and Research Laboratories.

Reprint of Dr. Meneely's article: "A Simplified Closed Circuit Helium Dilution Method, etc.," appearing in this issue, available on request.



CAMBRIDGE PULMONARY FUNCTION TESTER



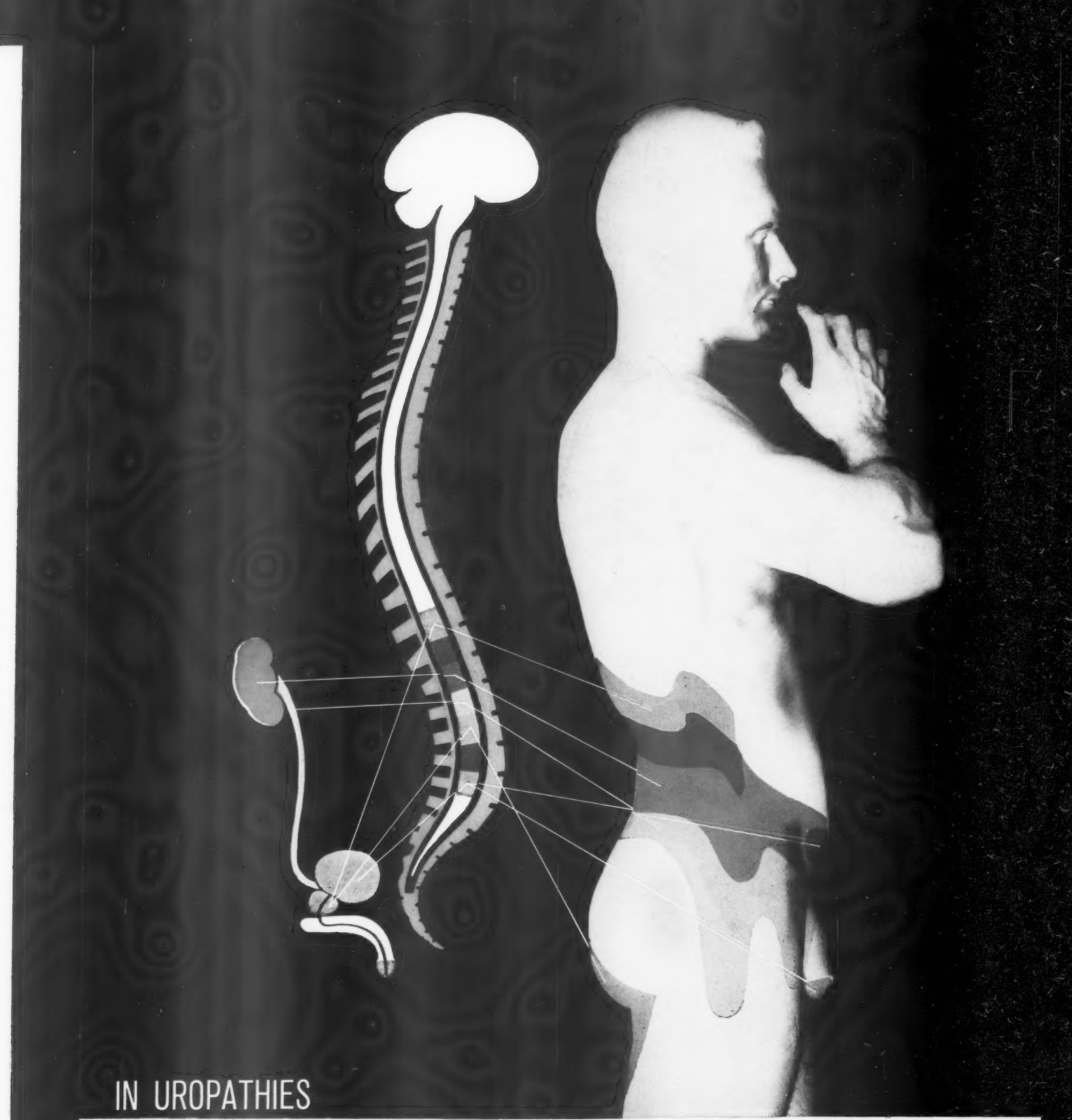
SCHEMATIC DIAGRAM OF THE TESTER

CAMBRIDGE INSTRUMENT CO., INC.

Graybar Bldg., 420 Lexington Ave., New York 17, N. Y.

Cleveland 2, Ohio	Oak Park, Ill.	Detroit 2, Mich.	Jenkintown, Pa.	Silver Spring, Md.
8419 Lake Avenue	6605 West North Avenue	7410 Woodward Avenue	479 Old York Road	933 Gist Avenue

PIONEER MANUFACTURERS OF THE ELECTROCARDIOGRAPH



IN UROPATHIES

WHEN PAIN IS PART OF THE PICTURE

Urinary tract pain, at the source or referred, is subject to the rapid analgesic action of the azo dye in Azo Gantrisin. Azo Gantrisin combines dramatic relief of symptoms with proven effective action against infections carried by either blood stream or urine.

Valuable also following urologic manipulation and surgery.

AZO GANTRISIN

GANTRISIN®—brand of sulfisoxazole ROCHE®

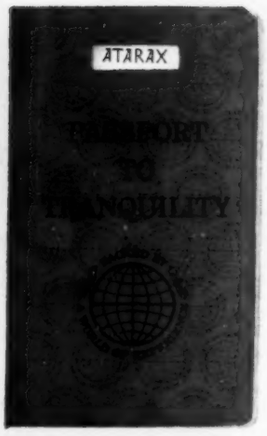
Diagram correlates sources of primary urinary pain with **areas of referred pain.**

Dosage: Adults—2 tablets four times daily.
Children under 100 lbs—1 tablet four times daily.
Each tablet provides 0.5 Gm Gantrisin plus 50 mg phenylazodiamino-pyridine HCl—bottles of 100 and 500.

ROCHE
LABORATORIES
Division of Hoffmann-La Roche Inc.
Nutley 10, N. J.

A GUIDE TO

THE REALMS OF THERAPY BEST ATTAINED WITH



ATARAX®

(brand of hydroxyzine)

World-wide record of effectiveness—over 200 laboratory and clinical papers from 14 countries.
Widest latitude of safety and flexibility—no serious adverse clinical reaction ever documented.
Chemically distinct among tranquilizers—not a phenothiazine or a meprobamate.
Added frontiers of usefulness—antihistaminic; mildly antiarrhythmic; does not stimulate gastric secretion.

Special Advantages	Supportive Clinical Observation	...and for additional evidence
IN CHILDREN unusually safe; tasty syrup, 10 mg. tablet	"... Atarax appeared to reduce anxiety and restlessness, improve sleep patterns and make the child more amenable to the development of new patterns of behavior...." Freedman, A. M.: Pediat. Clin. North America **5**:573 (Aug.) 1958.	Bayart, J.: Acta paediat. belg. **10**:164, 1956. Ayd, F. J., Jr.: California Med. **87**:75 (Aug.) 1957. Nathan, L. A., and Andelman, M. B.: Illinois M. J. **112**:171 (Oct.) 1957.
IN ELDERLY PATIENTS well tolerated by debilitated patients	"... seems to be the agent of choice in patients suffering from removal disorientation, confusion, conversion hysteria and other psychoneurotic conditions occurring in old age." Smigel, J. O., et al.: J. Am. Geriatrics Soc. **7**:61 (Jan.) 1959.	Settel, E.: Am. Pract. & Digest Treat. **8**:1584 (Oct.) 1957. Negri, F.: Minerva med. **48**:607 (Feb. 21) 1957. Shalowitz, M.: Geriatrics **11**:312 (July) 1956.
IN ALLERGIC PATIENTS useful adjunctive therapy for asthma and dermatosis; particularly effective in urticaria	"All [asthmatic] patients reported greater calmness and were able to rest and sleep better ... and led a more normal life.... In chronic and acute urticaria, however, hydroxyzine was effective as the sole medicament." Santos, I. M., and Unger, L.: Presented at 14th Annual Congress, American College of Allergists, Atlantic City, New Jersey, April 23-25, 1958.	Eisenberg, B. C.: J.A.M.A. **169**:14 (Jan. 3) 1959. Coirault, R., et al.: Presse méd. **64**:2239 (Dec. 26) 1956. Robinson, H. M., Jr., et al.: South. M. J. **50**:1282 (Oct.) 1957.
IN HYPEREMOTIVE ADULTS does not impair mental acuity	"... especially well-suited for ambulatory neurotics who must work, drive a car, or operate machinery." Ayd, F. J., Jr.: New York J. Med. **57**:1742 (May 15) 1957.	Garber, R. C., Jr.: J. Florida M. A. **45**:549 (Nov.) 1958. Menger, H. C.: New York J. Med. **58**:1684 (May 15) 1958. Farah, L.: Internat. Rec. Med. **169**:379 (June) 1956.

SUPPLIED: Tablets, 10 mg., 25 mg., 100 mg.; bottles of 100. Syrup (10 mg. per tsp.), pint bottles. Parenteral Solution: 25 mg./cc. in 10 cc. multiple-dose vials; 50 mg./cc. in 2 cc. ampules.

Roerig

New York 17, N. Y.
Division, Chas. Pfizer & Co., Inc.
Science for the World's Well-Being

A NEW CLASS OF DRUG FOR THE RELIEF OF PAIN

analexin™

phenyramidol HCl

the first analgomylaxant *a single chemical that is both a general non-narcotic analgesic and an effective muscle relaxant*

IN ABDOMINAL AND PELVIC PAIN

analexin

relieves the total pain experience

Analexin (phenyramidol HCl) is a new class of drug . . . the first analgomylaxant . . . a single chemical that inherently possesses two different pharmacologic actions within one molecular structure: i.e. (1) *general analgesia*, by raising the pain threshold and thus decreasing perception of pain, and (2) *muscle relaxation*, by selectively depressing subcortical and spinal polysynaptic transmission (interneuronal blockade), abolishing abnormal muscle tone without impairing neuromuscular function.[1-4] Thus, in painful states Analexin abolishes the pain and the muscle tensions which often augment it and manages the total pain experience more effectively.

with remarkably few side effects

Analexin is not related to any currently available analgesic or muscle relaxant drug. The analgesic potency of one tablet is clinically equivalent to 1 grain of codeine, yet Analexin is neither narcotic nor is it narcotic-related. It is not habituating. There is no evidence suggestive of tolerance or cumulative effects. Muscle relaxant action is comparable to the most potent oral muscle relaxants available.[3,4] The incidence of side effects is low and those reactions that have been reported (gastrointestinal irritation, pruritus) are of a mild and transient nature and do not limit therapy.[5]

Analexin ... ***for relief of pain and muscle tension.*** Each tablet contains 200 mg. of phenyramidol HCl. Dosage—1 or 2 tablets every 4 hours.

Analexin-AF ... ***for relief of pain and muscle tension complicated by fever and/or inflammation.*** Each tablet contains 100 mg. of phenyramidol HCl and 300 mg. of aluminum aspirin. Dosage—2 tablets every 4 hours or as required.

typical results with Analexin* and Analexin-AF in abdominal and pelvic pain[5-8]

Condition	No. Patients	Relief	Slight or no relief
pain associated with:			
duodenal ulcer	10	9	1
hiatus hernia	1	1	
gallbladder colic	5	2	3
dysmenorrhea	96	88	8
abdominal distress (flatulence, colic, constipation, etc.)	10	6	4
epigastric distress (pylorospasm, gastritis, etc.)	10	7	3
genitourinary pain	3	3	
pelvic pain (chronic P.I.D., endometriosis, etc.)	12	8	4
postpartum pain	100	100	
Totals	247	224	23

*Generically designated as phenyramidol HCl in clinical trials

typical comments from investigators

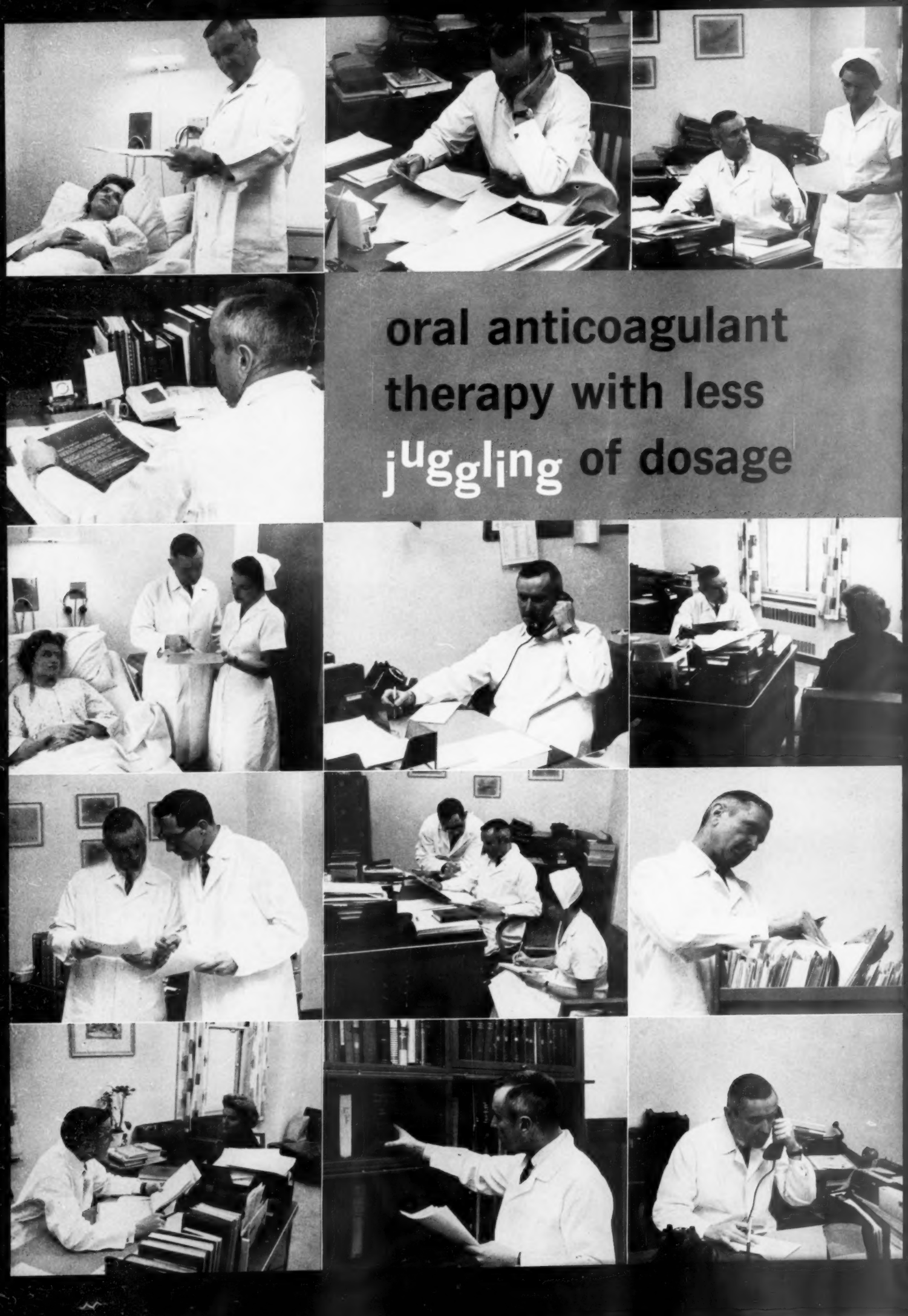
"Not only is satisfactory relief of painful states achieved in the majority of patients regardless of etiology and duration of pain, but there is also no evidence suggestive of cumulative toxicity. Furthermore, in contrast to codeine and meperidine, the likelihood of untoward reactions occurring in ambulant patients is not high."[5]

When phenyramidol with aspirin was used to replace aspirin and codeine, ". . . Codeine grains ½ with aspirin grains 10. Its clinical effectiveness was exactly the same as the former two agents combined."[6]

The patients with duodenal ulcer ". . . presented an excellent symptomatic control of their complaints. These patients were previously under treatment with antacids, antispasmodics and sedatives with recurrent pain and unsatisfactory control. Phenyramidol usually administered alone but occasionally with an antacid resulted in control of 9 of the 10 patients."[7]

REFERENCES: 1. O'Dell, T. B., *et al.*: J. Pharmacol. & Exper. Therap. *128*:65, 1960. 2. O'Dell, T. B., *et al.*: Fed. Proc. *18*:694, 1959. 3. Gray, A. P., and Heitmeier, D. E.: J. Am. Chem. Soc. *81*:4347, 1959. 4. Gray, A. P., *et al.*: J. Am. Chem. Soc. *81*:4351, 1959. 5. Batterman, R. C., *et al.*: Am. J. Med. Sc. *238*:315, 1959. 6. 511:5912, Clinical Data from the files of the Medical Department, Irwin, Neisler & Co., 1959. 7. Batterman, R. C.: Paper presented at the New York Academy of Sciences Symposium on "Non-narcotic Drugs for the Relief of Pain," Dec. 4, 1959. 8. Wainer, A. S.: Paper presented at the New York Academy of Sciences Symposium, Dec. 5, 1959.

Neisler IRWIN, NEISLER & CO. DECATUR, ILLINOIS


oral anticoagulant
therapy with less
juggling of dosage

introducing

PANWARFIN™

(Warfarin Sodium, Abbott)

for the prevention and treatment of intravascular thrombosis and embolism

The physician's great advantage with PANWARFIN is this: *he can establish stable oral dosage with relative simplicity.*

PANWARFIN is predictable in its effect. The physician will note but little day-to-day fluctuation in his patients' prothrombin times. He isn't beset by the usual need for frequently readjusting dosage. Guided by simple lab determinations, he gains early control of coagulability, and maintains the dosage with a minimum of tinkering.

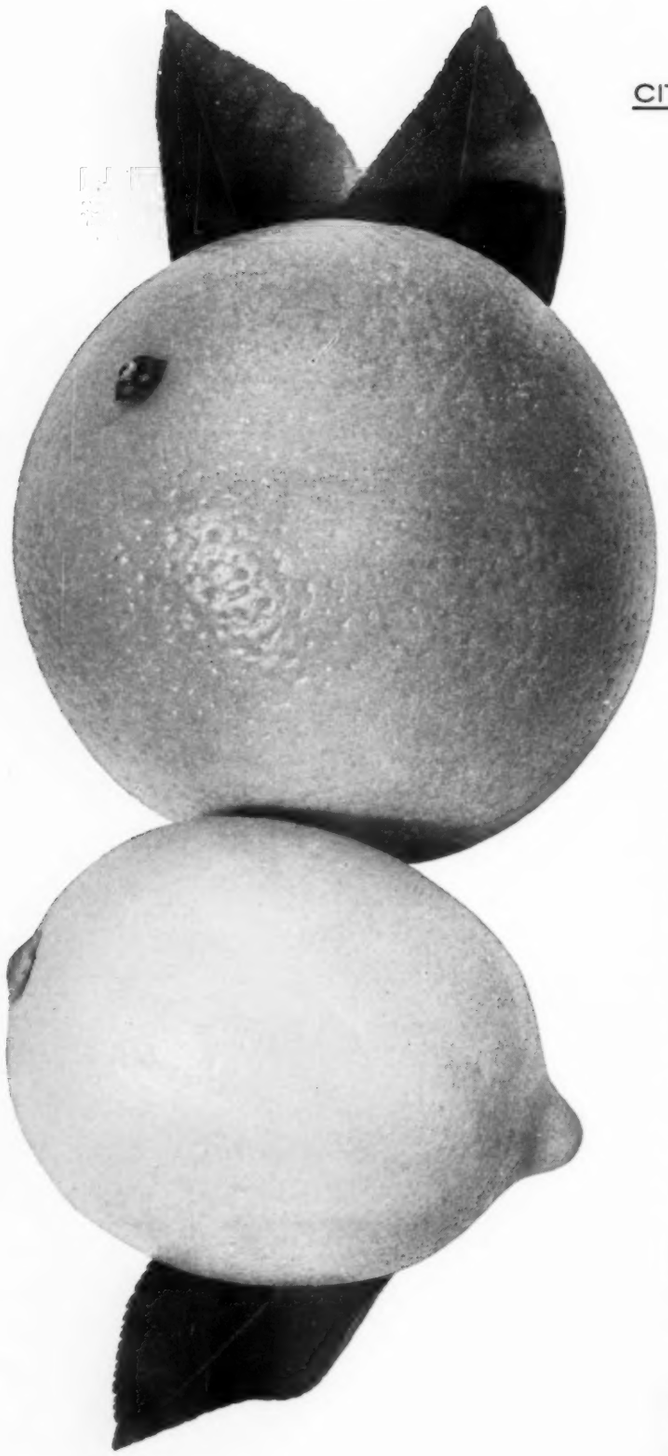
The initial dose provides therapeutic prothrombin levels within about 18 hours. Or, if *immediate* effect is desired, PANHEPRIN™ (Heparin Sodium, Abbott) may be given intravenously at the same time; after 24 hours, hypoprothrombinemia is then maintained by regular oral doses of PANWARFIN alone.

Consider PANWARFIN for your future anticoagulant regimens, doctor. Our literature gives full details. Ask your Abbott representative for it, or write.

SUPPLIED in 5-mg. white grooved tablets, List No. 6973, bottles of 100 and 1000; 10-mg. yellow grooved tablets, List No. 6988, bottles of 100 and 1000; and 25-mg. orange grooved tablets, List No. 6994, bottles of 25, 100, and 1000.

ABBOTT LABORATORIES NORTH CHICAGO, ILL.

003212



CITRUS BIOFLAVONOIDS

When abnormal cellular metabolism accompanies stress conditions

Hesperidin, Hesperidin Methyl Chalcone, or *Lemon Bioflavonoid Complex* are prescribed as therapeutic adjuncts for control of abnormal cellular activity, and capillary and vascular damage associated with many stress conditions.

These stress conditions may be caused by nutritional deficiencies, environment, drugs, chemicals, toxins, virus or infection.

SUNKIST AND EXCHANGE BRAND *Lemon Bioflavonoid Complex* and *Hesperidins* are available to the medical profession in specialty formulations developed by leading pharmaceutical manufacturers.

Sunkist Growers
PRODUCTS SALES DEPARTMENT
PHARMACEUTICAL DIVISION
Ontario, California

Control of Habitual Abortion

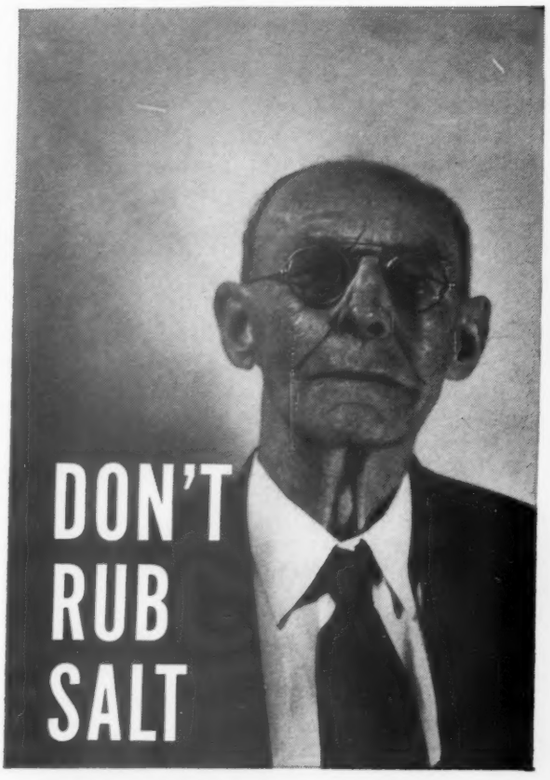
Disturbed capillary permeability and lowered capillary resistance, as well as the tendency toward edema and fluid retention, are well recognized in pregnancy (1, 2, 3, 4). The bioflavonoids have been shown effective in controlling the susceptibility to edema in pregnancy (5) and their routine prenatal use has been suggested (6).

Ecchymotic areas resulting from bruises and positive capillary fragility tests have frequently been observed in habitual aborters (7). Patients having a history of two or more spontaneous abortions have shown a marked improvement in fetal salvage after the addition of *Hesperidin* (a citrus bioflavonoid), ascorbic acid and other factors to the therapeutic regimen (8, 9, 12, 14, 15, 16). Other investigators have reported extensive use of the citrus bioflavonoids in the management of pregnancy with excellent results (18, 19, 20).

Observations include a reduction in severity or prevention of erythroblastosis fetalis in Rh-negative patients when *Hesperidin* (7) or other citrus bioflavonoids (23, 24) were administered.

The rationale of *Hesperidin* and other citrus bioflavonoids—in conjunction with vitamin C, nutritional factors or other therapeutic agents—as adjuncts in the management of pregnancy and its complications, spontaneous abortion and erythroblastosis fetalis, is based on the premise and observation that capillary involvement may be a contributing factor.

NOTE: For bibliography (B-688) write Sunkist Growers, Pharmaceutical Division, 720 East Sunkist Street, Ontario, California.



IN HIS CARDIAC WOUND

Modern saluretics may *seem* to have made unlimited salt intake possible for cardiac and hypertensive patients. Yet despite the improvements in diuretic therapy, sodium restriction is still important in the prophylaxis of edema. The wise physician does not add needlessly to the burden of his patient, nor test unnecessarily the power of the drugs he prescribes. It makes good sense to him to prescribe DIASAL—which looks, tastes and flavors food exactly like salt . . . but is sodium free.

Diasal contains potassium chloride, glutamic acid and inert ingredients. Supplied in shakers and 8 oz. bottles.

prescribe DIASAL®
sodium-free salt substitute

FOUGERA

E. Fougera & Co., Inc. • Hicksville, New York

When panic strikes your preoperative patient

VISTARIL, as part of a preoperative regimen, can safely relax your patients by allaying fear and apprehension. They are able to sleep soundly at night, and usually remain calm but alert during the day. Postoperatively, VISTARIL quiets anxiety and controls emesis.

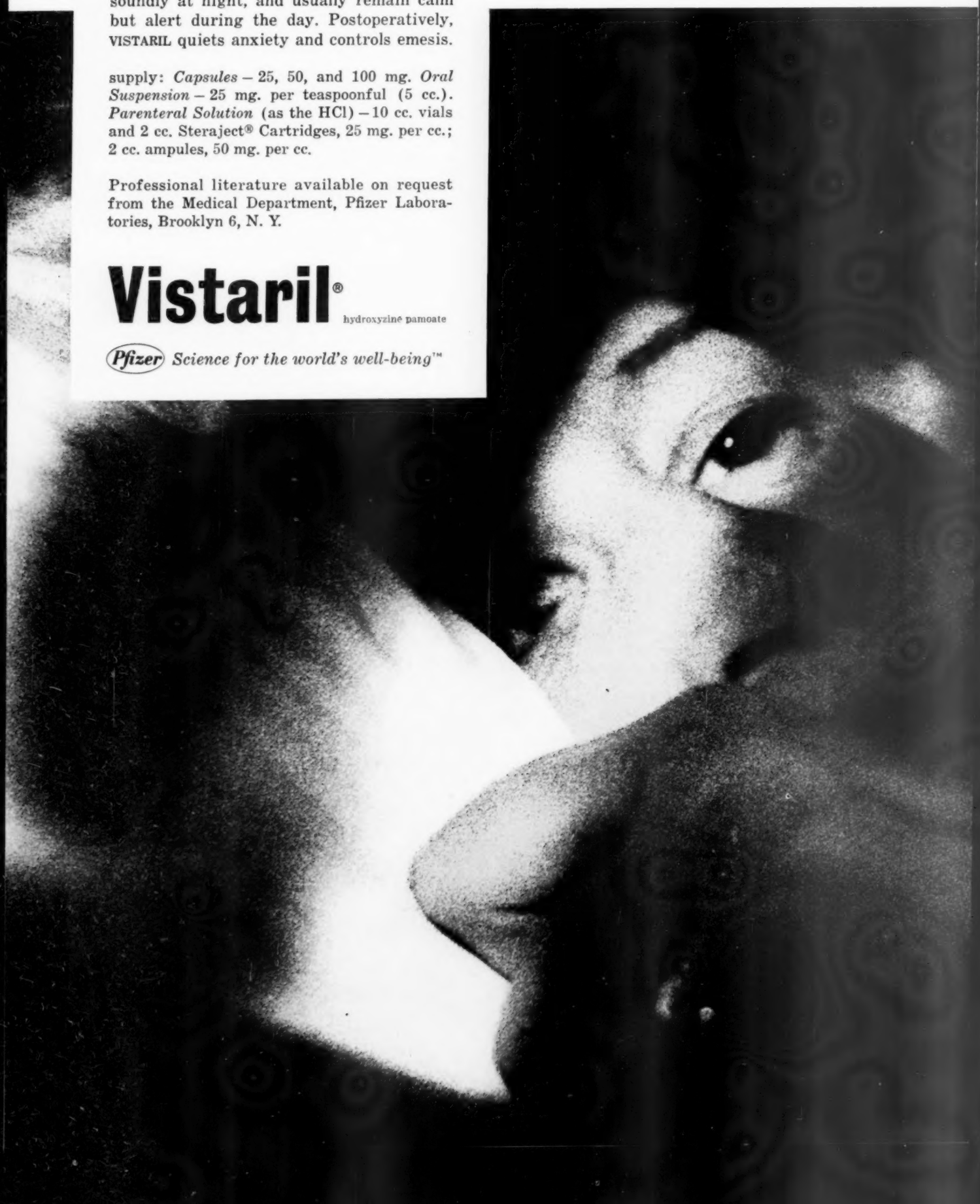
supply: *Capsules* – 25, 50, and 100 mg. *Oral Suspension* – 25 mg. per teaspoonful (5 cc.). *Parenteral Solution* (as the HCl) – 10 cc. vials and 2 cc. Steraject® Cartridges, 25 mg. per cc.; 2 cc. ampules, 50 mg. per cc.

Professional literature available on request from the Medical Department, Pfizer Laboratories, Brooklyn 6, N. Y.

Vistaril®

hydroxyzine pamoate

Pfizer *Science for the world's well-being™*



New Dianabol converts protein to working weight in wasting or debilitated patients

Mrs. M. R., 75-year-old underweight patient:

Puts on 13¼ needed pounds in just 6 weeks;

gains 1" on right biceps;

forces mercury column 14 mm. higher in cuff-compression test of muscle strength;

feels better than she has in 2 years.

Photos used with permission of the patient.

Dianabol is a new tissue-building agent with distinct advantages over previous compounds of this type.

By aiding the deposition, synthesis, and utilization of protein, Dianabol affords these benefits in the underweight elderly patients with or without serious disease and in patients who are chronically ill or convalescent:

- Rebuilds tissue and improves appetite, thus promoting lean weight gain.
- Restores tone to weak, flabby musculature.
- Speeds healing of wounds; hastens postoperative recovery and convalescence from a variety of diseases.
- Strengthens skeletal structure; often relieves pain and increases mobility in osteoporosis.
- Improves general physical status; helps to revive a sense of well-being.

Economical, convenient to administer, and almost without virilizing effects, Dianabol overcomes the disadvantages that have restricted use of tissue-building compounds in the past. Older patients, whose funds are often limited, will particularly welcome the low cost of Dianabol therapy—in most cases only 9 to 17 cents a day.

Complete information available on request.

SUPPLIED: *Tablets,* 5 mg. (pink, scored); bottles of 100.

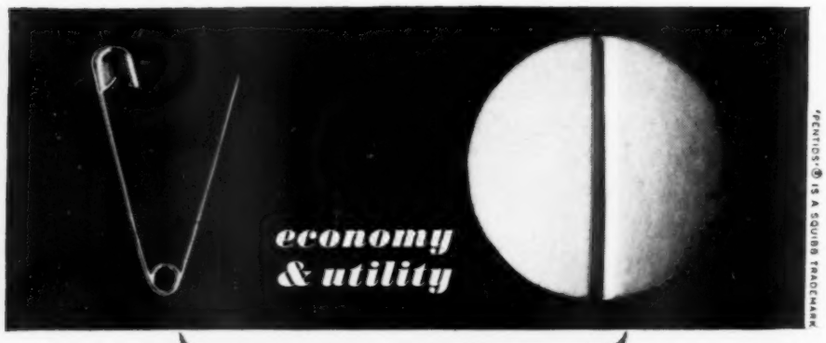
Dianabol®
(methandrostenolone CIBA)

New, orally effective tissue builder

Converts protein to working weight in wasting or debilitated patients

CIBA
SUMMIT, NEW JERSEY

2/2768MB



clinically proved
oral penicillin therapy
that costs your
patients less

Pentids

Squibb Penicillin G Potassium

SQUIBB

Squibb Quality—the Priceless Ingredient

Available in these convenient dosage forms: PENTIDS '400' TABLETS (400,000 u.) • PENTIDS '400' FOR SYRUP (400,000 u. per 5 cc. when prepared) • PENTIDS TABLETS (200,000 u.) • PENTIDS FOR SYRUP (200,000 u. per 5 cc. when prepared) • PENTID-SULFAS TABLETS (200,000 u. with 0.5 Gm. triple sulfas) • PENTIDS CAPSULES (200,000 u.) • PENTIDS SOLUBLE TABLETS (200,000 u.)

Back Issues Wanted

(MUST BE IN GOOD CONDITION)

THE AMERICAN JOURNAL OF MEDICINE

will pay $1.00 per copy for
the following issues:

May 1957 — December 1957

August 1957 — May 1958

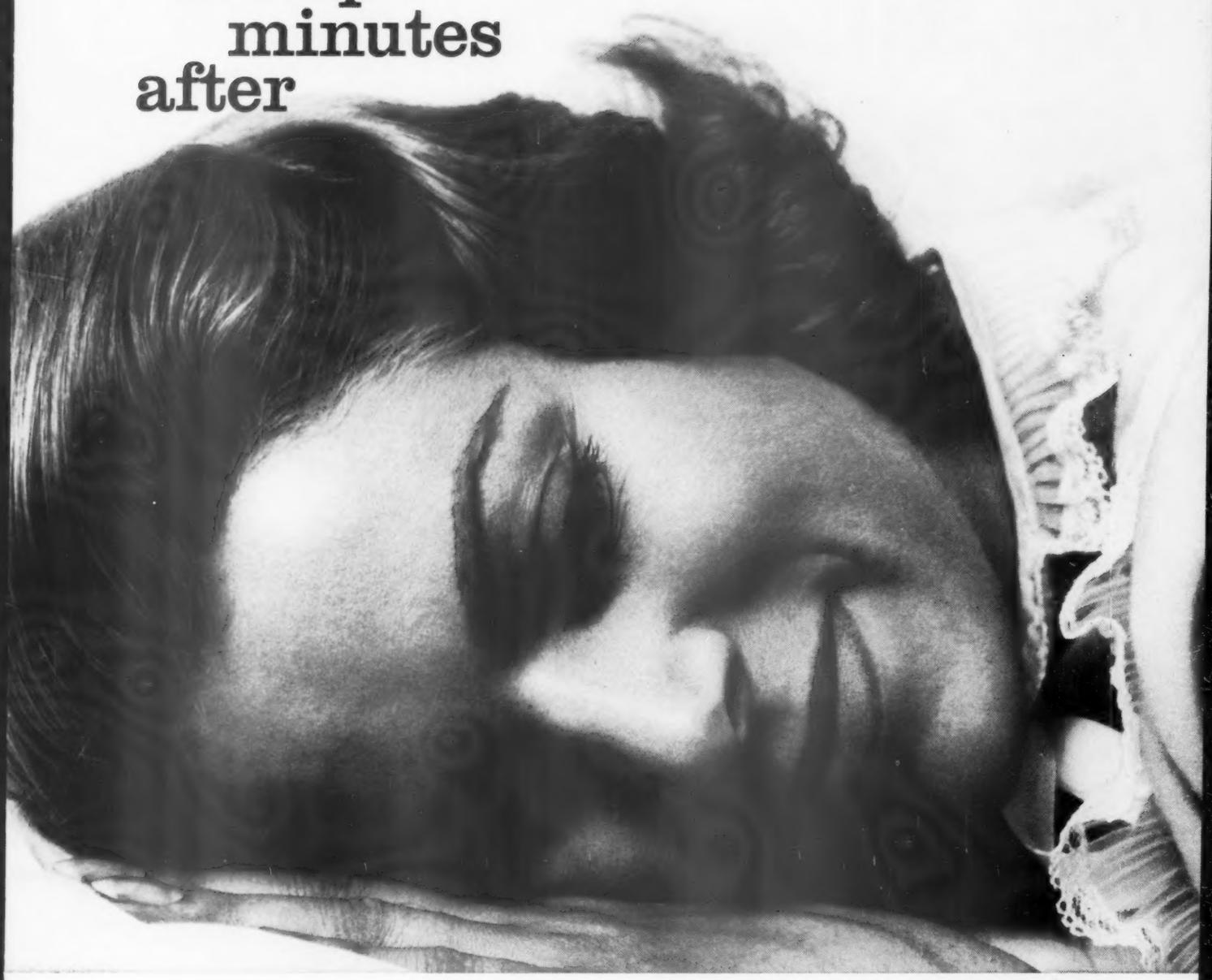
November 1957 — August 1958

October 1958

Send Postpaid to

The American Journal of Medicine, Inc.
11 East 36th Street, New York 16, N. Y.

asleep minutes after



This is the promise of Noludar 300... a night of deep, refreshing sleep without risk of habituation or toxicity... 6 to 8 hours of undisturbed rest... an easy awakening in the morning, free of fogginess or barbiturate "hangover." Try Noludar 300 for your next patient with a sleep problem. One capsule at bedtime. Chances are she'll tell you

"I slept like a log"

NOLUDAR® 300

brand of methyprylon

300-mg capsules

ROCHE LABORATORIES • Division of Hoffmann-La Roche Inc • Nutley 10, New Jersey

Lifts depression...

Your patient responds within a few days. Thanks to your prompt treatment and the smooth action of Deprol, his depression is relieved and his anxiety calmed – *often in two or three days.* He eats properly, sleeps well, and his depression no longer complicates your basic regimen.

as it calms anxiety!

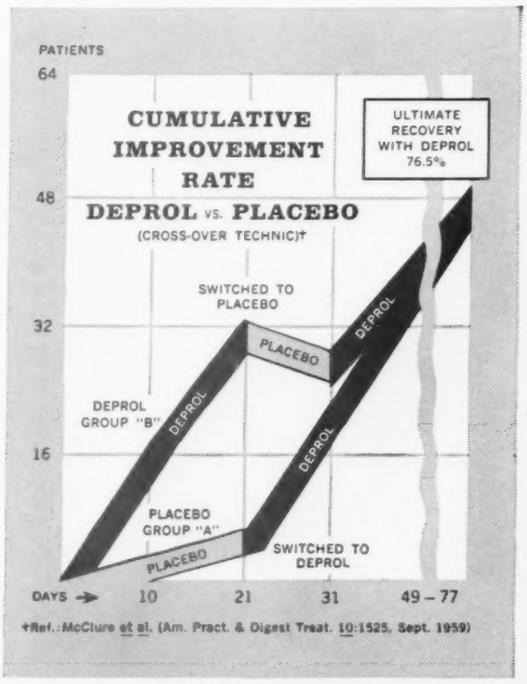
For cardiovascular and G.I. patients – a smooth, balanced action that lifts depression as it calms anxiety... rapidly and safely

Balances the mood – no "seesaw" effect of amphetamine-barbiturates and energizers. While amphetamines and energizers may stimulate the patient – *they often aggravate anxiety and tension.* And although amphetamine-barbiturate combinations may counteract excessive stimulation – *they often deepen depression.*

In contrast to such "seesaw" effects, Deprol lifts depression as it calms anxiety–both at the same time.

Acts swiftly – the patient often feels better, sleeps better, within two or three days. Unlike the delayed action of most other antidepressant drugs, which may take two to six weeks to bring results, Deprol relieves the patient quickly – often within two or three days.

Acts safely–no danger of hypotension or liver damage. Deprol does not cause liver toxicity, hypotension, tachycardia, jitteriness, vomiting, constipation or psychotic reactions frequently reported with other antidepressant drugs. It can be safely administered with basic therapy.



Results of a controlled study of 128 patients conducted by General Practitioners, Internists, Gastroenterologists, Urologists, Surgeons, Proctologists and others in collaboration with Psychiatrists.

▲Deprol▲®

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this may be gradually increased up to 3 tablets q.i.d.
Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.
Supplied: Bottles of 50 light-pink, scored tablets. Write for literature and samples.

WALLACE LABORATORIES/*New Brunswick, N. J.*

BIBLIOGRAPHY *(11 clinical studies, 764 patients):*

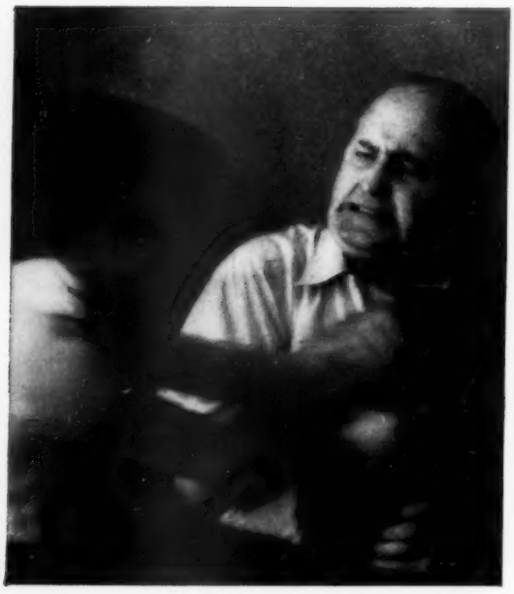
1. Alexander, L. (35 patients): Chemotherapy of depression – Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. **2.** Bateman, J. C. and Carlton, H. N. (50 patients): Meprobamate and benactyzine hydrochloride (Deprol) as adjunctive therapy for patients with advanced cancer. Antibiotic Med. & Clin. Therapy 6:648, Nov. 1959. **3.** Bell, J. L., Tauber, H., Santy, A. and Pulito, F. (77 patients): Treatment of depressive states in office practice. Dis. Nerv. System 20:263, June 1959. **4.** Breitner, C. (31 patients): On mental depressions. Dis. Nerv. System 20:142, (Section Two), May 1959. **5.** Landman, M. E. (50 patients): Choosing the right drug for the patient. Submitted for publication, 1960. **6.** McClure, C. W., Papas, P. N., Speare, G. S., Palmer, E., Slattery, J. J., Konefal, S. H., Henken, B. S., Wood, C. A. and Ceresia, G. B. (128 patients): Treatment of depression–New technics and therapy. Am. Pract. & Digest Treat. 10:1525, Sept. 1959. **7.** Pennington, V. M. (135 patients): Meprobamate-benactyzine (Deprol) in the treatment of chronic brain syndrome, schizophrenia and senility. J. Am. Geriatrics Soc. 7:656, Aug. 1959. **8.** Rickels, K. and Ewing, J. H. (35 patients): Deprol in depressive conditions. Dis. Nerv. System 20:364, (Section One), Aug. 1959. **9.** Ruchwarger, A. (87 patients): Use of Deprol (meprobamate combined with benactyzine hydrochloride) in the office treatment of depression. M. Ann. District of Columbia 28:438, Aug. 1959. **10.** Settel, E. (52 patients): Treatment of depression in the elderly with a meprobamate-benactyzine hydrochloride combination (Deprol). Antibiotic Med. & Clin. Therapy 7:28, Jan. 1960. **11.** Splitter, S. R. (84 patients): The care of the anxious and the depressed. Submitted for publication, 1959.

CD-762

announcing "Timovan"®

Brand of Prothipendyl hydrochloride

completely new calmative



for the temperamental older patient

for the emotional teen-ager

Ayerst®

6024

"Timovan"®

Brand of Prothipendyl hydrochloride

notably in geriatrics and adolescents

Specifically developed for active patients in need of calming without the "slow-down" of sedatives or the hazards of many tranquilizers. "TIMOVAN" offers a new range of safety and effectiveness in the relief of tension in the ambulatory patient, notably the adolescent and the geriatric. Particularly valuable in conditions in which excessive emotional response complicates therapy, as in dermatoses and allergies.[1-4]

- Reduces excessive response to irritating stimuli.
- Stabilizes the autonomic nervous system.
- Nonhypnotic, yet improves sleep pattern.
- No sensitivity reactions or toxicity reported.
- Has not given rise to drug tolerance even on prolonged use.
- Nonaddictive.
- Preferred to barbiturates.

AYERST LABORATORIES
New York 16, N. Y. • Montreal, Canada

DOSAGE: One or two tablets three or four times daily. Depending on age of patient and severity of symptoms, dosages ranging from 100 mg. to 400 mg. daily (in divided doses) have been used effectively and safely.

CONTRAINDICATIONS: Not to be used in cases of acute alcoholism or barbiturate poisoning.

SUPPLIED: "TIMOVAN" No. 739 — 25 mg. tablets. No. 740 — 50 mg. tablets. Bottles of 100 and 1,000.

REFERENCES: 1. Medical Records of Chemiewerk Homburg A/G. 2. Linke, H.: München. med. Wchnschr. **100**:969 (June 20) 1958. 3. Quandt, V. J., Von Horn, L., and Schliep, H.: Psychiat. et Neurol. **135**:197 (Mar.) 1958. 4. Medical Records of Ayerst Laboratories.

Many **MIGRAINE** attacks can be **stopped at the start** by the prompt use of...

'MIGRAL'®

Advantage

'MIGRAL' permits maximum ergotamine therapy with the first dose —because the 'MIGRAL' formula includes the proved antiemetic, cyclizine hydrochloride, to counteract the tendency to nausea and vomiting.

Dosage

'MIGRAL' should be taken immediately at the start of a migraine attack, and the effective dosage should be determined on an individual basis. When the total dosage necessary to stop an attack has been determined, that amount should be taken as initial dosage in subsequent attacks.

In general, 2 to 4 'MIGRAL' tablets taken at the first sign of an attack will terminate a headache by preventing progression to the vasodilation stage. If treatment is not started sufficiently early to achieve this result, an additional 1 or 2 tablets should be administered every half hour until the patient is relieved, or until a total dosage of 6 tablets has been taken.

Caution

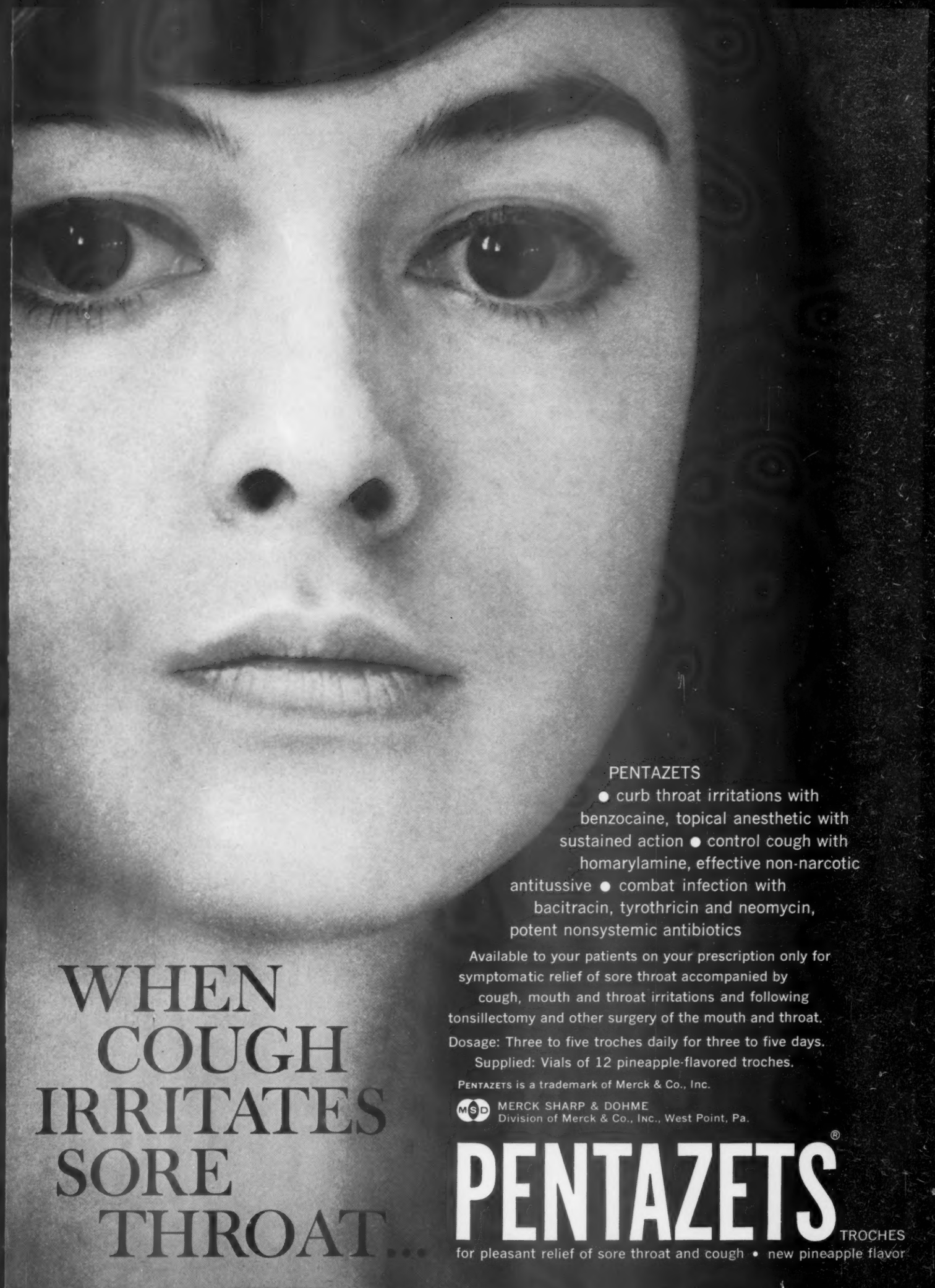
It is recommended that not more than 6 tablets be taken during a single attack, nor more than 10 tablets per week.

Each sugar-coated 'MIGRAL' tablet provides:

Ergotamine Tartrate	1 mg.
'Marezine'® brand Cyclizine Hydrochloride	25 mg.
Caffeine	50 mg.

In bottles of 20 and 100 tablets.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York



WHEN COUGH IRRITATES SORE THROAT...

PENTAZETS
● curb throat irritations with benzocaine, topical anesthetic with sustained action ● control cough with homarylamine, effective non-narcotic antitussive ● combat infection with bacitracin, tyrothricin and neomycin, potent nonsystemic antibiotics

Available to your patients on your prescription only for symptomatic relief of sore throat accompanied by cough, mouth and throat irritations and following tonsillectomy and other surgery of the mouth and throat.

Dosage: Three to five troches daily for three to five days.

Supplied: Vials of 12 pineapple-flavored troches.

PENTAZETS is a trademark of Merck & Co., Inc.

MSD MERCK SHARP & DOHME
Division of Merck & Co., Inc., West Point, Pa.

PENTAZETS®

TROCHES

for pleasant relief of sore throat and cough • new pineapple flavor

Naturetin

Squibb Benzydroflumethiazide

Naturetin c̄ K

Squibb Benzydroflumethiazide with Potassium Chloride

"...a safe and extraordinarily effective diuretic..."[1]

Naturetin – reliable therapy in edema and hypertension – maintains a favorable urinary sodium-potassium excretion ratio . . . retains a balanced electrolytic pattern:

"...the increase in urinary output occurs promptly..."[1]

"...the least likely to invoke a negative potassium balance..."[2]

"...a dose of 5 mg. of Naturetin produces a maximal sodium loss."[2]

"...an effective diuretic agent as manifested by the loss in weight..."[3]

"...no apparent influence of clinical importance on the serum electrolytes or white blood count."[3]

"...no untoward reactions were attributed to the drug."[4]

Although Naturetin causes the least serum potassium depletion as compared with other diuretics, supplementary potassium chloride in Naturetin c̄ K provides added protection when treating hypokalemia-prone patients; in conditions where likelihood of electrolyte imbalance is increased or during extended periods of therapy.

Numerous clinical studies confirm the effectiveness[1-15] of Naturetin as a diuretic and antihypertensive – usually in dosages of 5 mg. per day.

■ the most potent diuretic, mg. for mg.—more than 100 times as potent as chlorothiazide ■ prolonged action – in excess of 18 hours ■ maintains its efficacy as a diuretic and antihypertensive even after prolonged or increased dosage use ■ convenient once-a-day dosage – more economical for patients ■ low toxicity – few side effects—low sodium diets not necessary ■ not contraindicated except in complete renal shutdown ■ *in hypertension*—significant lowering of the blood pressure. Naturetin may be used alone or with other antihypertensive drugs in lowered doses.

Supplied: Naturetin Tablets, 5 mg. (scored) and 2.5 mg. Naturetin c̄ K (5 c̄ 500) Tablets (capsule-shaped) containing 5 mg. benzydroflumethiazide and 500 mg. potassium chloride. Naturetin c̄ K (2.5 c̄ 500) Tablets (capsule-shaped) containing 2.5 mg. benzydroflumethiazide and 500 mg. potassium chloride.

SQUIBB

Squibb Quality—the Priceless Ingredient

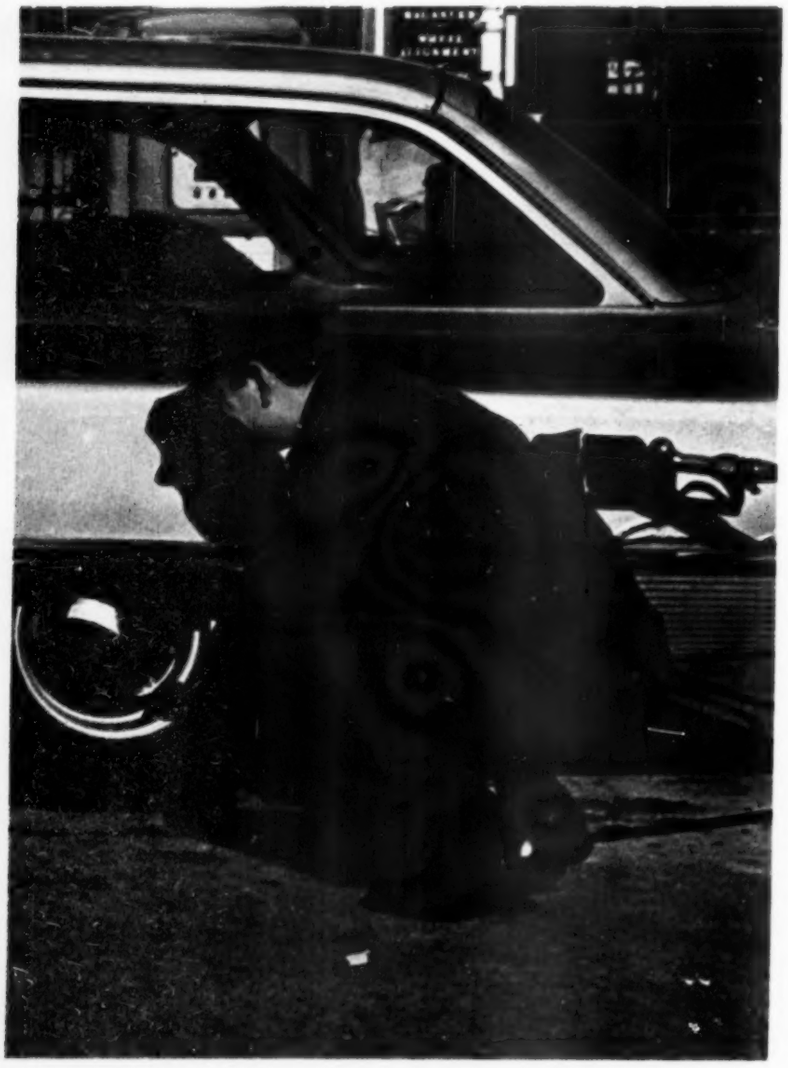
References: 1. David, N. A.; Porter, G. A., and Gray, R. H.: Monographs on Therapy 5:60 (Feb.) 1960. 2. Stenberg, E. S., Jr.; Benedetti, A., and Forsham, P. H.: Op. cit. 5:46 (Feb.) 1960. 3. Fuchs, M.; Moyer, J. H., and Newman, B.E.: Op. cit. 5:55 (Feb.) 1960. 4. Marriott, H. J. L., and Schamroth, L.: Op. cit. 5:14 (Feb.) 1960. 5. Ira, G. H., Jr.; Shaw, D. M., and Bogdonoff, M. D.: North Carolina M. J. 21:19 (Jan.) 1960. 6. Cohen, B. M.: M. Times, to be published. 7. Breneman, G. M., and Keyes, J. W.: Henry Ford Hosp. M. Bull. 7:281 (Dec.) 1959. 8. Forsham, P. H.: Squibb Clin. Res. Notes 2:5 (Dec.) 1959. 9. Larson, E.: Op. cit. 2:10 (Dec.) 1959. 10. Kirkendall, W. M.: Op. cit. 2:11 (Dec.) 1959. 11. Yu, P. N.: Op. cit. 2:12 (Dec.) 1959. 12. Weiss, S.; Weiss, J., and Weiss, B.: Op. cit. 2:13 (Dec.) 1959. 13. Moser, M.: Op. cit. 2:13 (Dec.) 1959. 14. Kahn, A., and Greenblatt, I. J.: Op. cit. 2:15 (Dec.) 1959. 15. Grollman, A.: Monographs on Therapy 5:1 (Feb.) 1960.

'NATURETIN' IS A SQUIBB TRADEMARK.

Clinical reports on LOW BACK PAIN show that *Trancopal*® a true "tranquilaxant," keeps the patient on the job

A TRUE "TRANQUILAXANT"

Trancopal®



relaxes skeletal muscle spasm so the patient can continue to work

Clinical experience shows that Trancopal will enable your patients with low back pain to keep going strong. Lichtman[1] reports that 310 of his 331 patients treated with Trancopal obtained satisfactory relief. These patients were suffering from low back pain, stiff neck, postoperative muscle spasm or other skeletal muscle spasms associated with trauma, bursitis, osteoarthritis and rheumatoid arthritis. Mullin and Epifano[2] reported that Trancopal brought relief to all of 39 patients with skeletal muscle spasm. In these patients, who had suffered from trauma, bursitis, rheumatoid arthritis, osteoarthritis, and intervertebral disc syndrome, the effect of Trancopal was ". . . excellent and prompt . . ."[2] Gruenberg[3] obtained marked relief with Trancopal in 258 of 304 patients with low back pain, torticollis, arthritis and other conditions associated with skeletal muscle spasm. Moderate relief was obtained in an additional group of 28 patients. Trancopal is a true "tranquilaxant" because "It combines the properties of tranquilization and skeletal muscle relaxation with no concomitant change in normal consciousness."[4] Side effects have been few and minor — and in no case were they serious enough to warrant discontinuing the use of Trancopal.[1] "Trancopal is exceptionally safe for clinical use."[3]

relieves anxiety and tension so the patient can carry on

Trancopal is also an effective agent for patients in anxiety and tension states. According to recent clinical reports,[1,5] it calms the patients but allows them to continue their work or other activity. Indeed, Lichtman found that his patients with anxiety ". . . were in many instances able to continue their normal activities where previously they had been considerably restricted . . ."[1] He observed that Trancopal brought good to excellent relief to 114 of 120 patients in anxiety states. Ganz,[5] who noted good to excellent relief in 32 of 35 patients with globus hystericus, and in his entire series of 100 patients in anxiety or tension states, comments: "Chlormethazanone [Trancopal], by relieving the psychogenic symptoms, allows the patient to use his energies in a more productive manner in overcoming his basic problems."[5]

Relieves dysmenorrhea — Trancopal has also proved to be a useful medication in the treatment of patients with dysmenorrhea,[1,4,6] probably producing its effect ". . . by means of a combination of muscle relaxant and tranquilizing actions."[4]

Indications

Musculoskeletal disorders		Psychogenic disorders
Low back pain (lumbago)	Ankle sprain, tennis elbow	Dysmenorrhea
Neck pain (torticollis)	Osteoarthritis	Premenstrual tension
Bursitis	Rheumatoid arthritis	Anxiety and tension states
Fibrositis	Disc syndrome	Asthma
Myositis	Postoperative muscle spasm	Angina pectoris
		Alcoholism

Dosage: Adults, 100 or 200 mg. orally three or four times daily. Relief of symptoms generally occurs promptly and lasts from four to six hours.

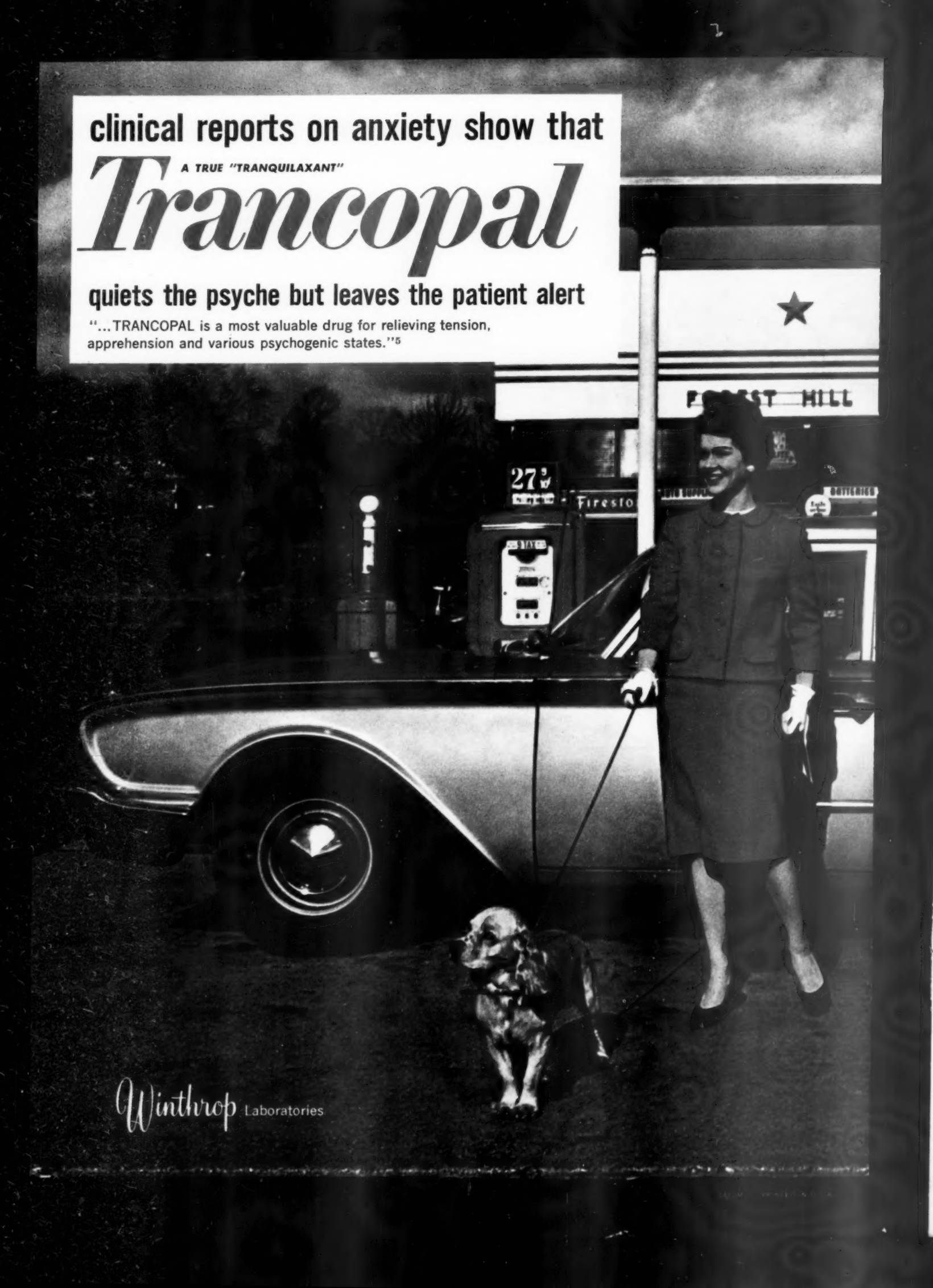
How Supplied: Trancopal Caplets® 100 mg. (peach colored, scored) and 200 mg. (green colored, scored), bottles of 100.

References: 1. Lichtman, A. L.: Kentucky Acad. Gen. Pract. J. 4:28, Oct., 1958. 2. Mullin, W. G., and Epifano, Leonard: Am. Pract. & Digest Treat. 10:1743, Oct., 1959. 3. Gruenberg, Friedrich: Current Therap. Res. 2:1, Jan., 1960. 4. Shanaphy, J. F.: Current Therap. Res. 1:59, Oct., 1959. 5. Ganz, S. E.: J. Indiana M. A. 52:1134, July, 1959. 6. Stough, A. R.: J. Oklahoma M. A. 52:575, Sept., 1959.

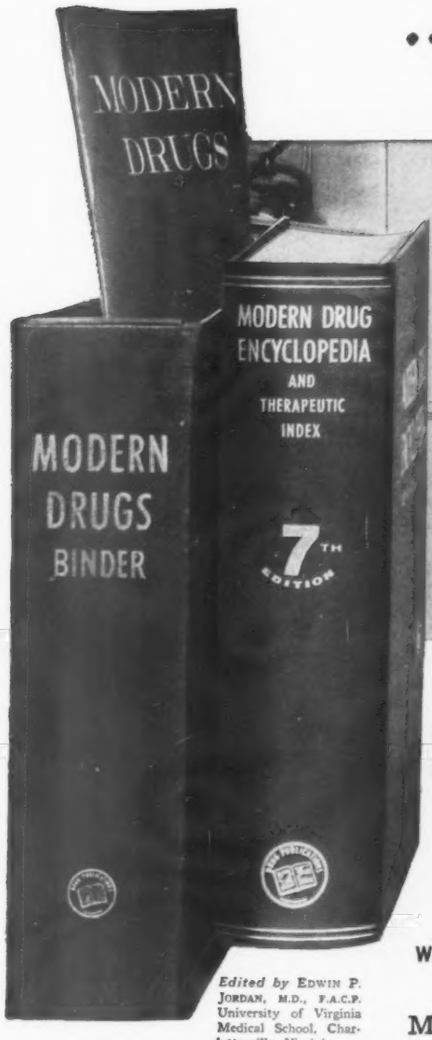
Winthrop Laboratories • New York 18, New York

PROFESSIONAL MODELS USED FOR PHOTOGRAPHS

TRANCOPAL (BRAND OF CHLORMEZANONE) AND CAPLETS, TRADEMARKS REG. U. S. PAT. OFF.


clinical reports on anxiety show that
A TRUE "TRANQUILAXANT"
Trancopal
quiets the psyche but leaves the patient alert
"...TRANCOPAL is a most valuable drug for relieving tension, apprehension and various psychogenic states."5
Winthrop Laboratories

..."my indispensable source for new drug descriptions"



Edited by EDWIN P. JORDAN, M.D., F.A.C.P. University of Virginia Medical School, Charlottesville, Virginia.

7th Edition

MODERN DRUG ENCYCLOPEDIA

AND THERAPEUTIC INDEX

with FREE 3-YEAR bi-monthly supplement service, MODERN DRUGS

More than 70% of the prescriptions written today call for new drugs introduced within the past 3 years. It's imperative that your drug reference be complete, dependable, current and up-to-date. Here is your authority on PRESCRIPTION DRUGS and the *new* NARCOTIC CLASSIFICATIONS—your up-to-the-minute source for latest composition or description, action, use, supply, dosage, caution and administration data on more than 4,000 drugs.

★ INDEXED to save you time. Therapeutic, Drug, Manufacturer's and new Generic Name Indices plus self-pronouncing drug listings.

★ COMPLETE, authoritative, continuing service for 3 years—New 7th Edition plus 18 bi-monthly MODERN DRUGS Supplements—all for $17.50.

★ ATTRACTIVELY BOUND in durable red cover stock. Approx. 1500 pages. Size 6" x 9¼" x 2¼".

---------ORDER THIS 3-YEAR SERVICE TODAY--------

MODERN DRUGS M-5/60
11 East 36th Street, New York 16, New York

☐ Please send me the 7th Edition MODERN DRUG ENCYCLOPEDIA and Therapeutic Index, plus bi-monthly MODERN DRUGS Supplements for 3 years—all for $17.50*
☐ Send along Binder for MODERN DRUGS Supplements—$3.00
☐ Remittance Enclosed ☐ Bill me later

Name_____

Address_____

City_____ Zone_____ State_____

*$17.50 in U.S.A., Foreign, $21.00

you can often treat the underlying cause of chronic fatigue with

NIAMID®

brand of nialamide

the mood brightener

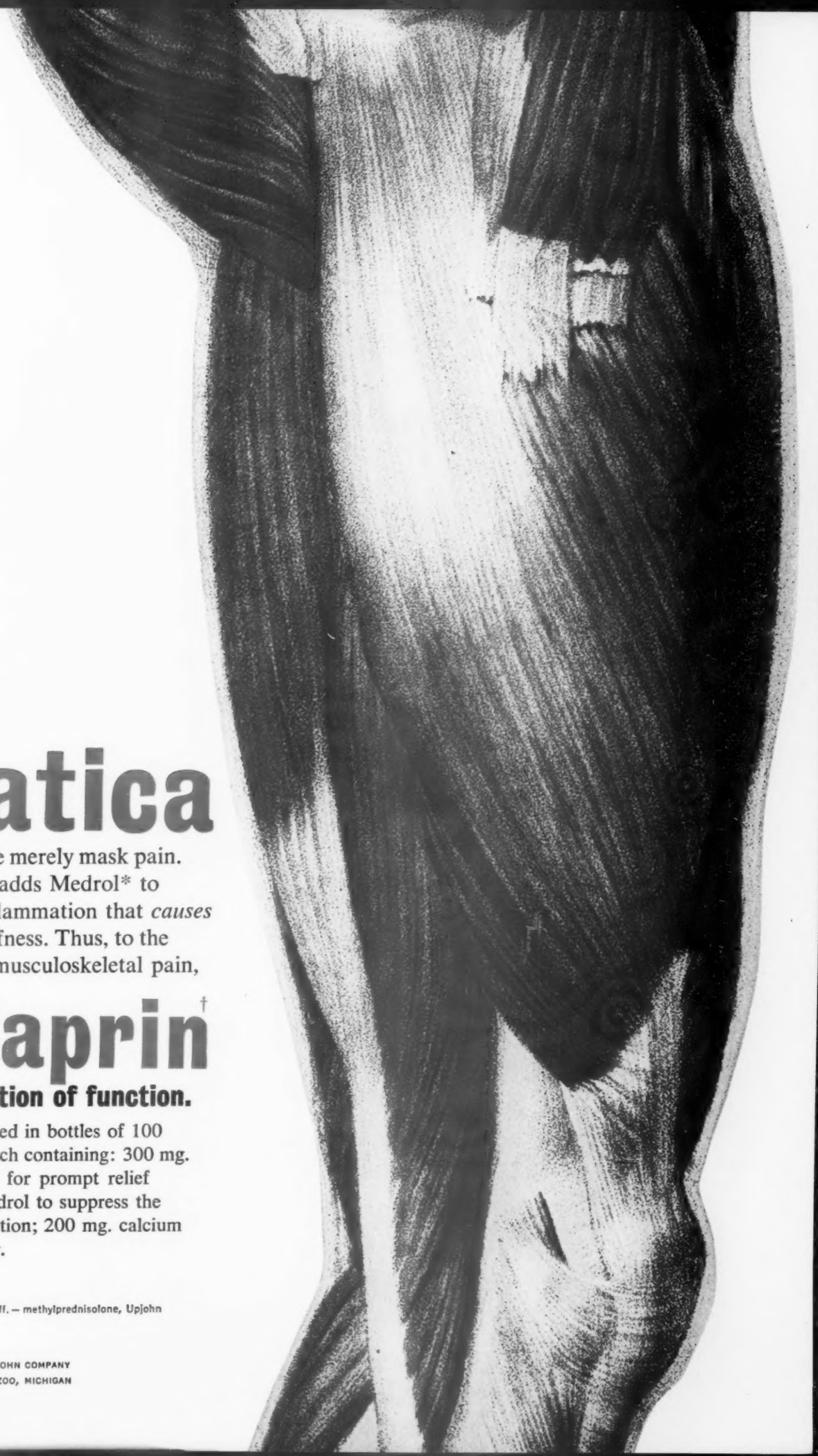
Depression becomes a prime suspect in chronic fatigue, once physical causes are ruled out. The patient who always wakes up tired, drags wearily through the day and comes home too exhausted to enjoy his family, may need NIAMID. After the mood-brightening action of NIAMID reduces the inertia of depression, many patients can work and play enthusiastically.

The effect of NIAMID on depression appears to be achieved by restoring neurohormone balance. The patient's family is usually first to notice his reawakening interest in life. The gradual, gentle action of NIAMID begins to renew a sense of well-being within a few days in some patients, and within two or three weeks in most other patients.

An exceptionally well tolerated antidepressant—more than 500,000 prescriptions in many clinical conditions—more than 90 published papers.

NIAMID is supplied as 25 and 100 mg. scored tablets. A Professional Information Booklet is available on request from the Medical Department, Pfizer Laboratories, Div., Chas. Pfizer & Co., Inc., Brooklyn 6, New York.

Pfizer *Science for the world's well-being™*



sciatica

Analgesics alone merely mask pain. New Medaprin adds Medrol* to suppress the inflammation that *causes* the pain and stiffness. Thus, to the direct relief of musculoskeletal pain,

Medaprin†

adds restoration of function.

Medaprin is supplied in bottles of 100 and 500 tablets, each containing: 300 mg. acetylsalicylic acid for prompt relief of pain; 1 mg. Medrol to suppress the causative inflammation; 200 mg. calcium carbonate as buffer.

*Trademark, Reg. U. S. Pat. Off. — methylprednisolone, Upjohn

†Trademark

Upjohn

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

NOW WIDELY PRESCRIBED QUINIDINE THERAPY IN CARDIAC ARRHYTHMIAS

Quinaglute T.M. dura-tab® s.m.

exclusive oral
Sustained Medication*
Quinidine Gluconate (5 gr.)

for these good reasons[1-4]

q. 12 h. dosage
QUINAGLUTE DURA-TAB S.M. tablets maintain effective uniform blood levels around the clock, day and night.

better tolerated
because quinidine gluconate is ten times more soluble than the sulfate — and easier on the g.i. tract.

uniformly efficient
no let-down in plasma levels where arrhythmias tend to occur.

Bottles of 30, 100 and 250.

PDR PAGE 893

1. Bellet, S., Finkelstein, D., and Gilmore, H.: A.M.A. Archives Int. Med. 100:750, 1957.
2. Bellet, S.: Amer. Heart J. 56:479, 1958.
3. Finkelstein, D.: Penn. Med. J. 61:1216, 1958.
4. Bellet, S.: Amer. J. Cardiology 4:268, 1959.

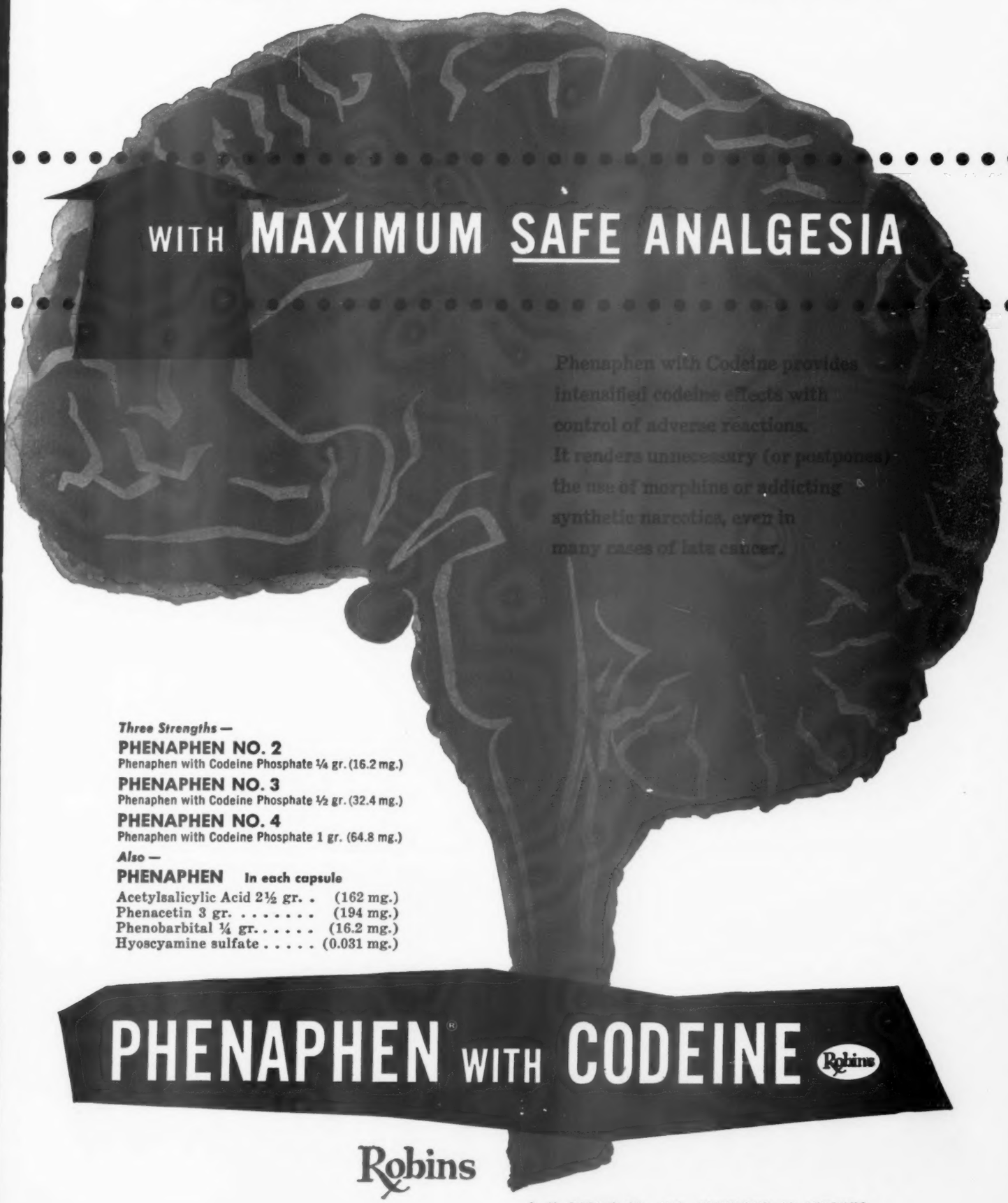
Samples and literature — write . . .

WYNN PHARMACAL CORPORATION
Lancaster Ave. at 51st Street, Philadelphia 31, Pa.

Also available: INJECTABLE QUINAGLUTE
10 cc. Vials, 0.08 Gm. Quinidine Gluconate per cc.

*U.S. Patent 2895881

Raise the Pain Threshold



WITH MAXIMUM SAFE ANALGESIA

Phenaphen with Codeine provides intensified codeine effects with control of adverse reactions. It renders unnecessary (or postpones) the use of morphine or addicting synthetic narcotics, even in many cases of late cancer.

Three Strengths —

PHENAPHEN NO. 2
Phenaphen with Codeine Phosphate ¼ gr. (16.2 mg.)

PHENAPHEN NO. 3
Phenaphen with Codeine Phosphate ½ gr. (32.4 mg.)

PHENAPHEN NO. 4
Phenaphen with Codeine Phosphate 1 gr. (64.8 mg.)

Also —

PHENAPHEN In each capsule

Acetylsalicylic Acid 2½ gr. . (162 mg.)
Phenacetin 3 gr. . . . . (194 mg.)
Phenobarbital ¼ gr. . . . . (16.2 mg.)
Hyoscyamine sulfate . . . . . (0.031 mg.)

PHENAPHEN® WITH CODEINE

Robins

A. H. ROBINS CO., INC., RICHMOND 20, VIRGINIA

Ethical Pharmaceuticals of Merit since 1878

An instructive new seminar
that presents recent clinical findings on

MYCOTIC INFECTIONS

This seminar contains:

Current Concepts of Diagnostic Serology and Skin Hypersensitivity in the Mycoses.

S. B. SALVIN, PH.D., *Hamilton, Montana.*

From the U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institutes of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana.

The Course and Prognosis of Histoplasmosis.

HARRY RUBIN, M.D., MICHAEL L. FURCOLOW, M.D., *Kansas City, Kansas,* J. LEWIS YATES, M.D. and CHARLES A. BRASHER, M.D., *Mount Vernon, Missouri.*

From the Kansas City Field Station, Communicable Disease Center, Bureau of State Services, Public Health Service, U.S. Department of Health, Education, and Welfare, University of Kansas School of Medicine, Kansas City, Kansas, and the Missouri State Sanatorium, Mount Vernon, Missouri.

Aspergillosis. A Review and Report of Twelve Cases.

SYDNEY M. FINEGOLD, M.D., DRAKE WILL, M.D. and JOHN F. MURRAY, M.D., *Los Angeles, California.*

From the Department of Medicine, Wadsworth Hospital, Veterans Administration Center, Los Angeles, and Department of Medicine and Pathology, University of California Medical Center, Los Angeles, California.

The Use of Amphotericin B in the Treatment of Coccidioidal Disease.

WILLIAM A. WINN, M.D., *Springville, California.*

From the Department of Medicine, Tulare-Kings Counties Hospital, Springville, California.

North American Blastomycosis.

E. RICHARD HARRELL, M.D. and ARTHUR C. CURTIS, M.D., *Ann Arbor, Michigan.*

From the Department of Dermatology, University of Michigan Medical Center, and the V.A. Hospital, Ann Arbor, Michigan.

Cryptococcosis (Torulosis). Current Concepts and Therapy.

M. L. LITTMAN, M.D., PH.D., *New York, New York.*

From the Departments of Microbioloby and Medicine, the Mount Sinai Hospital, New York, N. Y. These studies were supported in part by research grants from the Squibb Institute for Medical Research and the National Science Foundation.

Actinomycosis and Nocardiosis. A Review of Basic Differences in Therapy.

JOSEPH W. PEABODY, JR., M.D., *Washington, D.C.* and JOHN H. SEABURY, M.D., *New Orleans, Louisiana.*

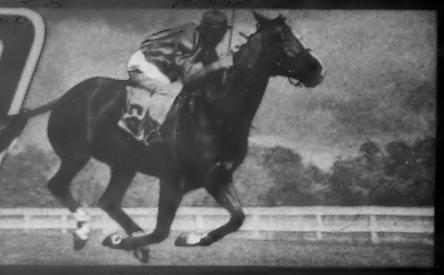
[Fully illustrated—Many references included]

The Seminar on Mycotic Infections originally appeared in The American Journal of Medicine and is now available in bound library reference form at $3.00 per copy.

THE AMERICAN JOURNAL OF MEDICINE

11 East 36th Street • New York 16, N. Y.

SPEED



DURATION

IN *ORAL* CONTROL OF PAIN

ACTS FASTER—usually within 5-15 minutes. **LASTS LONGER**—usually 6 hours or more. **MORE THOROUGH RELIEF**—permits uninterrupted sleep through the night. **RARELY CONSTIPATES**—excellent for chronic or bedridden patients.

AVERAGE ADULT DOSE: 1 tablet every 6 hours. May be habit-forming. Federal law permits oral prescription.

Each PERCODAN® Tablet contains 4.50 mg. dihydrohydroxycodeinone hydrochloride, 0.38 mg. dihydrohydroxycodeinone terephthalate, 0.38 mg. homatropine terephthalate, 224 mg. acetylsalicylic acid, 160 mg. phenacetin, and 32 mg. caffeine.

Also available — for greater flexibility in dosage — PERCODAN®-DEMI: The PERCODAN formula with one-half the amount of salts of dihydrohydroxycodeinone and homatropine.

Endo®

Literature? Write
ENDO LABORATORIES
Richmond Hill 18, New York

Percodan® *Tablets*

Salts of Dihydrohydroxycodeinone and Homatropine, plus APC

FOR PAIN

U.S. Pat. 2,628,185

containing Oxethazaine
a gastric mucosal
anesthetic

OXAINE*

Oxethazaine in Alumina Gel, Wyeth

for

gastritis

an original development,
backed by 5 years' research
and clinical trial

*Trademark

OXAINE contains a gastric mucosal anesthetic for the relief of pain of gastritis.

OXAINE is indicated in the many patients who do not respond to diet, antacids and anticholinergics.

As reported in J.A.M.A., OXAINE brought complete relief to 96% of 92 gastritis patients suffering substernal pain and upper abdominal distress.
Deutsch, E., and Christian, H. J.: J.A.M.A. 169:2012 *(April 25) 1959.*

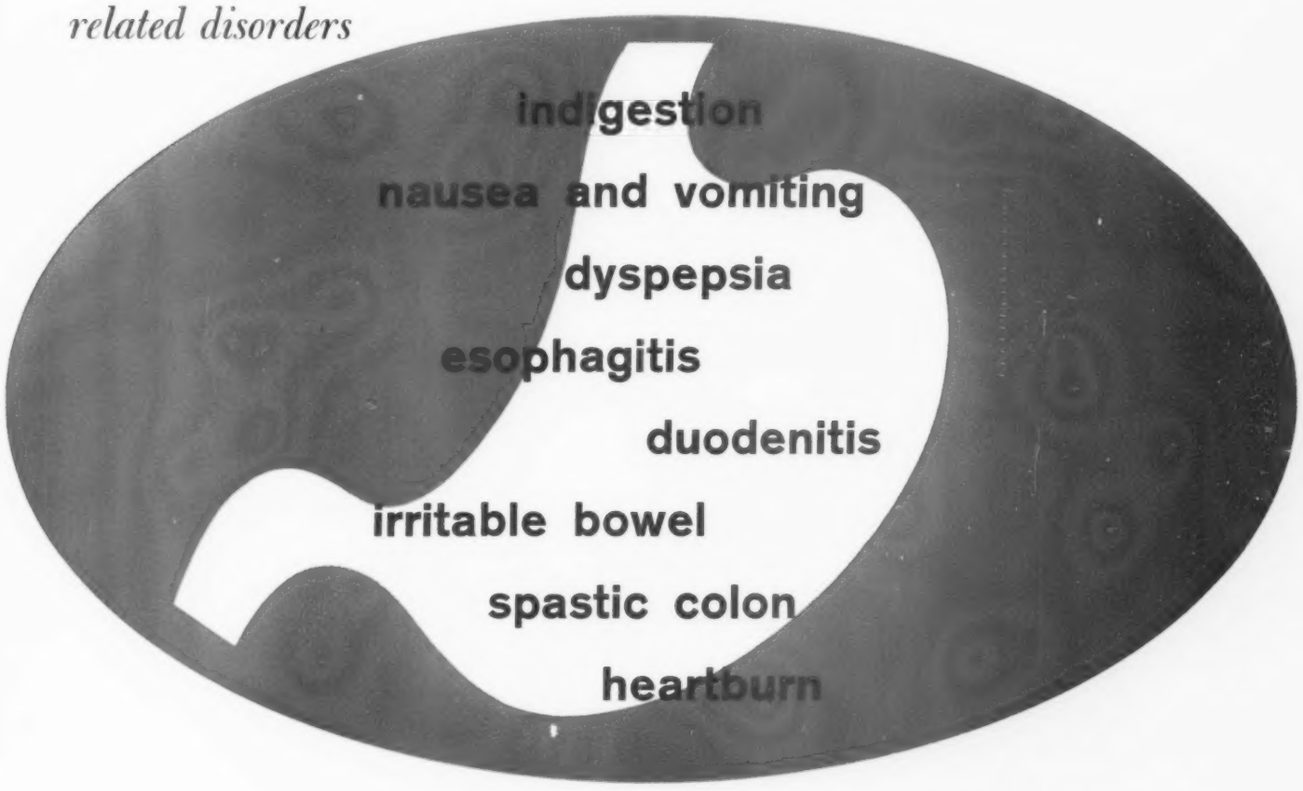
OXAINE provides sustained anesthesia over many hours, unaffected by ebb and flow of gastric contents.

Oxethazaine, the mucosal anesthetic in OXAINE, is 4000 times more potent topically than procaine. Safe, not a "caine." Only two known cases of sensitivity (glossitis) occurred in extensive clinical trials.

Easily administered, simple dosage—just 2 teaspoonfuls 15 minutes before meals and at bedtime.

Bland, noncloying over long-term administration.

related disorders



How OXAINE *Relieves Pain, Hastens Recovery*

Gastric mucosa can heal more quickly, because local anesthetics inhibit acid and pepsin secretion, by preventing release of *gastrin* from the antrum of the stomach.

Patients tolerate a more varied diet and a larger amount of food—and, because of OXAINE, enjoy their food without fear of pain following meals.

They feel free of bloating and the disturbing sensation of fullness when only a little food has been ingested—because the anesthesia of OXAINE desensitizes irritated nerve receptors.

Those with irritable bowel syndrome are spared the embarrassing urge to defecate during meals —because OXAINE diminishes the exacerbated gastrocolic reflex.

Supplied: *In bottles of 12 fluidounces.*

Wyeth Laboratories Philadelphia 1, Pa.

100 ANNIVERSARY Wyeth ®

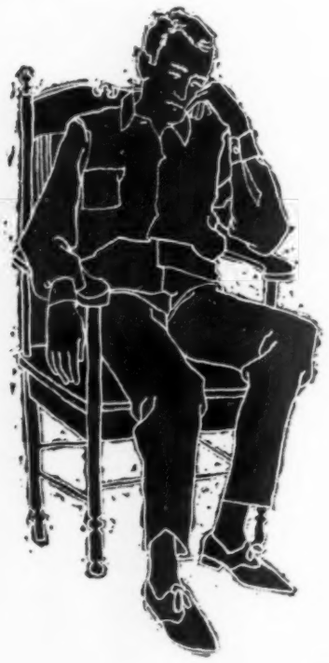
A Century of Service to Medicine

to help control
progressive disorders
of aging...

ELDEC®
mineral-vitamin-hormone supplement

begins at 40 **KAPSEALS®**

Taken during the middle years, ELDEC Kapseals help forestall nutritional and hormonal deficiencies that contribute to the troublesome disorders of aging. ELDEC Kapseals provide comprehensive physiologic supplementation...aid in maintaining metabolic efficiency. At a time when normal function is declining, ELDEC Kapseals help lay a firm foundation for good health and vitality in the later years.



PARKE, DAVIS & COMPANY
Detroit 32, Michigan

REPRINT ORDER FORM

THE AMERICAN JOURNAL
OF MEDICINE
11 E. 36th St., New York 16, N. Y.

Please send me the following reprints from THE AMERICAN JOURNAL OF MEDICINE.

☐ SYMPOSIUM ON RENAL PHYSIOLOGY	$4.00

SEMINARS

☐ ALLERGY	$2.00
☐ DISEASES OF THE PANCREAS	$2.00
☐ BONE DISEASES	$2.00
☐ LIVER DISEASE	$2.00
☐ MYCOTIC INFECTIONS	$3.00

Enclosed is my check

Name_____

Address_____

City_____ State_____

still available!

a limited number
of copies of

The Symposium

on

Diagnostic Enzymology

Price $4.00

This appeared in the
December Issue
of

The American Journal of Medicine
11 EAST 36TH STREET
NEW YORK 16, N. Y.

for happy,
healthy retirement years

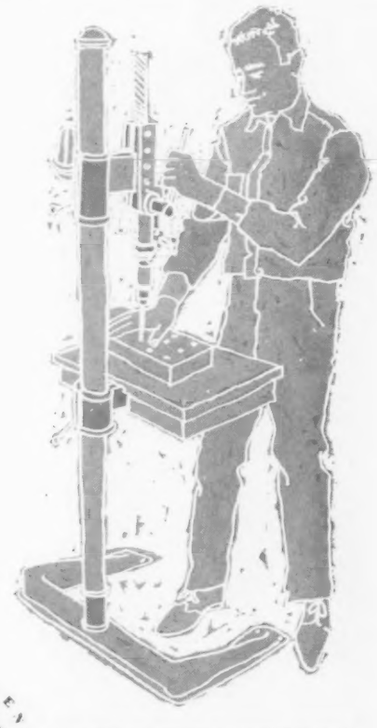
ELDEC®
comprehensive physiologic supplement

begins at 40 **KAPSEALS®**

Physiologic Prophylaxis

- *10 important vitamins plus minerals* to help maintain cellular function and correct deficiencies
- *protein improvement factors* to help compensate for unwise choice of food
- *digestive enzymes* to aid in offsetting decreased natural production
- *steroids* to stimulate metabolism and prevent or help correct protein depletion states

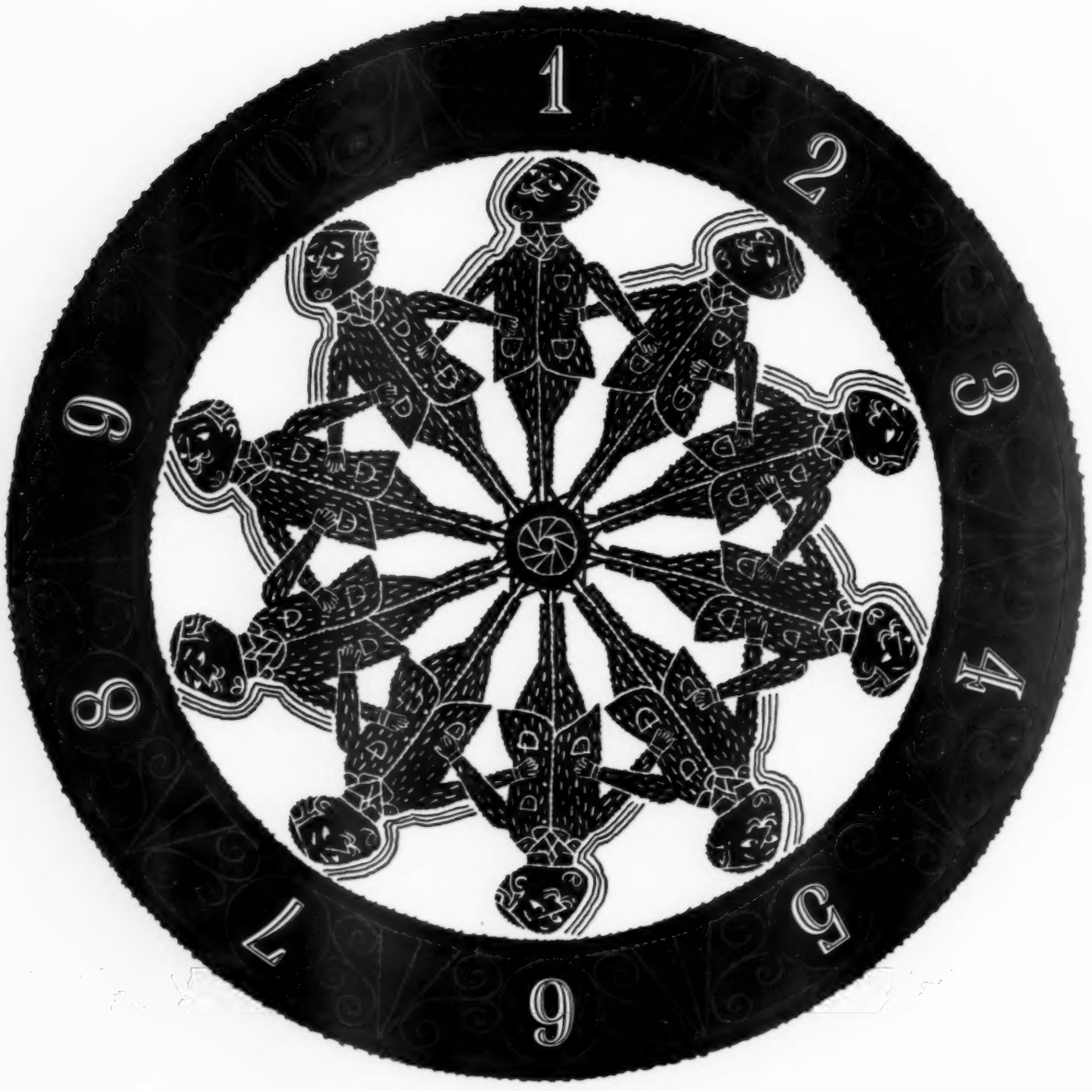
Packaging: ELDEC Kapseals are available in bottles of 100.



MEDICAMENTA VERA

PARKE, DAVIS & COMPANY
Detroit 32, Michigan

09360



Antivert®

STOPS VERTIGO 9 TIMES OUT OF 10!!

The latest ANTIVERT report confirms earlier findings: ANTIVERT relieves vertigo in 9 out of 10 patients. This combination of meclizine (an outstanding antihistamine for vestibular dysfunction) and nicotinic acid (the drug of choice for prompt vasodilation[1]) "... proved more effective than the use of either drug alone."[2] Out of 50 patients with Meniere's syndrome, only 4 failed to respond to ANTIVERT.[2]

Prescribe one ANTIVERT tablet (12.5 mg. meclizine; 50 mg. nicotinic acid) before each meal for relief of Meniere's syndrome, arteriosclerotic vertigo, labyrinthitis and vertigo of nonspecific origin.

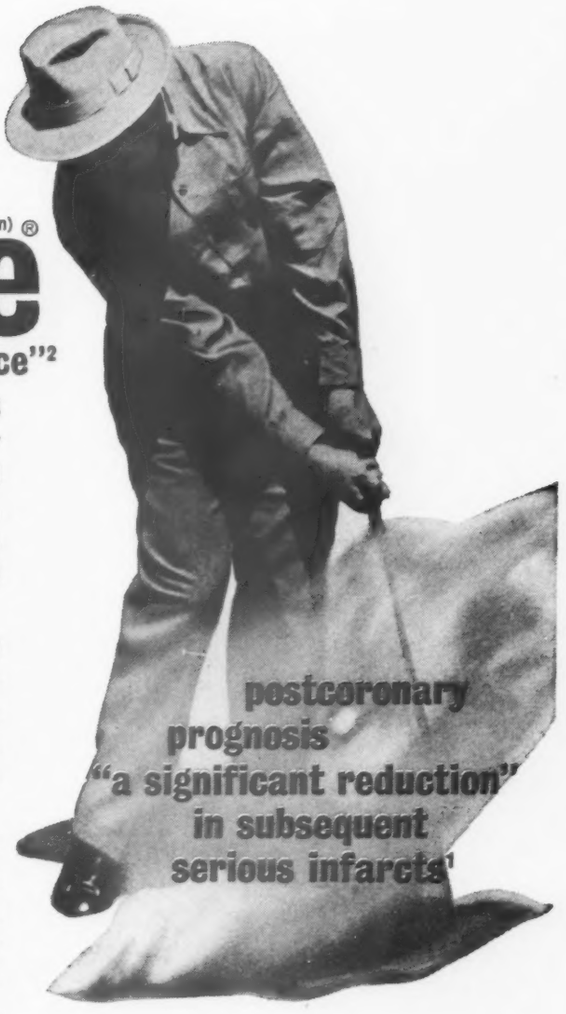
Supplied: **In bottles of 100 blue-and-white scored tablets. Prescription only.**

References: **1. Menger, H. C.: Clin. Med. 4:313 (Mar.) 1957. 2. Scal, J. C.: Eye Ear Nose & Throat Month. *38*:738 (Sept.) 1959.**

New York 17, N. Y.
Division, Chas. Pfizer & Co., Inc.
Science for the World's Well-Being

Danilone (Phenindione, Schieffelin) ®

"the anticoagulant drug of choice"[2]



When DANILONE was administered to 33 postmyocardial infarct patients, only two thromboembolic episodes occurred in two patients in 565 patient-months. Five such episodes in 50 patient-months occurred in four patients who had stopped taking anticoagulants.[3]

"twice as fast in reaching therapeutic levels"[4] In 117 patients, the average time DANILONE required to attain therapeutic levels was less than 36 hours.[4] With bishydroxycoumarin, two to four days are required for equal effect.[5]

"a greater degree of control"[6] DANILONE held prothrombin activity in 200 patients to between 5 and 10 per cent of normal "with greater facility" than in another series where bishydroxycoumarin was used.[6] Generally, most satisfactory control can be obtained by dividing the dosage schedule into two 12-hour intervals.[3,6]

"a reputation for safety"[7] DANILONE, unlike the coumarin derivatives, has a short recovery period and no cumulative action.[5,8,9] As a result, DANILONE "... can be administered with reasonable safety in the presence of many conditions which would contraindicate the use of Dicoumarol."[6]

"a lack of toxic reactions"[3] Because of its extremely low incidence of toxicity,[2,4,9] and "... the apparent safety factor of a wide spread between therapeutic and toxic dose, it is felt that phenyldanedione [phenindione] is the anticoagulant drug of choice."[2]

"inexpensive"[5] DANILONE is the least expensive anticoagulant available today. Maintenance cost is as little as three cents a day. In addition, its stability of effect permits fewer prothrombin-time tests,[9] saving the patient additional costs.

Samples and literature available on request.

SUPPLIED: 50 mg. scored tablets. Bottles of 100 and 1,000.

References: 1. Pickering, G., et al.: Brit. M. J. 1:803 (Mar. 28) 1959. 2. McCabe, E. S., and Fittipoldi, J., Jr.: Am. Pract. & Digest Treat. 4:765, 1953. 3. Drinan, F. W., et al.: Am. Heart J. 53:284, 1957. 4. Harper, B. F., and Johnson, R.: J.M.A. Georgia 45:149, 1956. 5. Wood, J. E.; Beckwith, J. R., and Camp, J. L., III: J.A.M.A. 159:635 (Oct. 15) 1955. 6. Sise, H. S.; Maloney, W. C., and Guttas, C. G.: Am. Heart J. 53:132, 1957. 7. Newcomb, T. F.: New England J. Med. 260:545 (Mar. 12) 1959. 8. O'Connor, W. R.; Thompson, C. E., and Baker, L. A.: Quart. Bull. Northwestern Univ. M. School 26:193 (Fall) 1952. 9. Toohey, M.: Brit. M. J. 1:650 (Mar. 21) 1953.

Schieffelin & Co. / Since 1794 Pharmaceutical Laboratories Division, New York 3

In the low back syndrome

relieves both stiffness and pain with safety... sustained effect

NOTABLE SAFETY—unusually low toxicity; no known contraindications: side effects are rare; drowsiness may occur, usually at higher dosage.

RAPID ACTION—starts to act quickly.

SUSTAINED EFFECT—relief lasts up to 6 hours.

EASY TO USE—usual adult dosage is one 350 mg. tablet 3 times daily and at bedtime.

SOMA T.M.

(carisoprodol Wallace)

Supplied
as white, coated, 350 mg. tablets, bottles of 50. Also available for pediatric use: *250 mg. orange capsules, bottles of 50.*

WALLACE LABORATORIES, *New Brunswick, New Jersey* *Literature and samples on request*

How to win little friends and influence recovery

Tastefully tailored to the antibiotic needs of pediatric patients

new Cosa-Terrabon*

oxytetracycline with glucosamine

Delicious in taste: the appealing flavor of sweet, fresh fruit

Decisive in action: the well-tolerated broad-spectrum efficacy of Terramycin® with glucosamine

Preconstituted for uniform potency, efficacy, and taste-appeal from the first dose to the last.

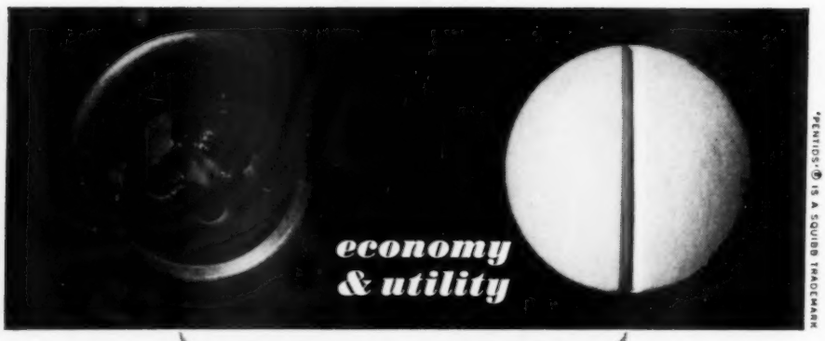
Cosa-Terrabon Oral Suspension – 125 mg. oxytetracycline/5 cc., 2 oz. and 1 pint bottles

Cosa-Terrabon Pediatric Drops – 100 mg. oxytetracycline/1 cc., 10 cc. bottle with plastic calibrated dropper

*Trademark

Pfizer Laboratories, Div., Chas. Pfizer & Co., Inc., Brooklyn 6, N. Y.

Pfizer *Science for the world's well-being™*



"PENTIDS"® IS A SQUIBB TRADEMARK

clinically proved oral penicillin therapy that costs your patients less

Pentids

Squibb Penicillin G Potassium

SQUIBB

Squibb Quality—the Priceless Ingredient

Available in these convenient dosage forms: PENTIDS '400' TABLETS (400,000 u.) • PENTIDS '400' FOR SYRUP (400,000 u. per 5 cc. when prepared) • PENTIDS TABLETS (200,000 u.) • PENTIDS FOR SYRUP (200,000 u. per 5 cc. when prepared) • PENTID-SULFAS TABLETS (200,000 u. with 0.5 Gm. triple sulfas) • PENTIDS CAPSULES (200,000 u.) • PENTIDS SOLUBLE TABLETS (200,000 u.)

CHANGING YOUR ADDRESS?

Please fill out and return the coupon below:

THE AMERICAN JOURNAL OF MEDICINE
Mailing Dept., 11 East 36th Street, New York 16, N. Y.

NAME_____
(please print)

NEW ADDRESS:

Street_____

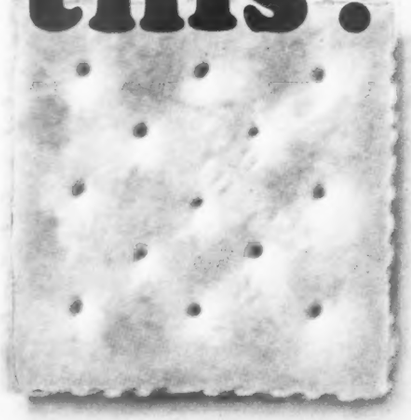
City_____ Zone_____ State_____

FORMER ADDRESS:

Street_____

City_____ Zone_____ State_____

...or this?



NEEDED: THE APPETITE SUPPRESSANT STRONG ENOUGH AND SAFE ENOUGH TO DO THE JOB

Ambar controls many cases of overeating/obesity refractory to usual therapy. To strengthen the will for successful dieting, the methamphetamine-phenobarbital in Ambar is designed to improve mood without harmful CNS overstimulation. Available in different forms to enable individualization of dosage: **AMBAR #1 EXTENTABS,** 10-12 hour extended action tablets, methamphetamine HCl 10.0 mg., phenobarbital 64.8 mg. **AMBAR #2 EXTENTABS,** methamphetamine HCl 15.0 mg., phenobarbital 64.8 mg. Also conventional **AMBAR TABLETS,** methamphetamine 3.33 mg., phenobarbital 21.6 mg.

Robins

A. H. ROBINS CO., INC., RICHMOND 20, VA.

Ambar™ #1 Extentabs®/Ambar™ #2 Extentabs®

if **you** were writing a paper
on the treatment of allergies...

your literature search would reveal that New DIMETANE is recommended antihistaminic therapy

HIGHLY EFFECTIVE / A.M.A. COUNCIL ON DRUGS J.A.M.A. 170:194, 1959 "... a high order of antihistaminic effectiveness and a low incidence of side effects."

SIDE REACTIONS AS FEW AS PLACEBO / NEW ENGLAND J. MED. 261:478, 1959 (Schiller, I. W., and Lowell, F. C.) "In contrast to the frequency of central-nervous-system-stimulating or sedative effects produced by chlorprophenpyridamine was the virtual freedom from these relatively disagreeable effects. ...Side effects in patients taking placebo were as frequent as those seen with parabromdylamine (Dimetane)."

NO PENALTY FOR ANTIALLERGIC POTENCY / ANNALS OF ALLERGY 17:19, 1959 (Lipman, W. H.) ". . . proved to be the safest antihistaminic agent that we have used in the treatment of disturbances due to allergies."

EXCELLENT PATIENT RESPONSE EVEN IN THOSE INTOLERANT OF OTHER ANTIHISTAMINES / ANNALS OF ALLERGY 16:128, 1958 (Thomas, J. W.) ". . . according to the patients' statements, this drug was found superior to previous antihistaminic therapy . . . in certain instances patients who were not able to tolerate other antihistamines tolerated Dimetane and obtained a satisfactory response."

"94.6%" EFFECTIVENESS RATE IN ALLERGIC AND PRURITIC DERMATOSES / ANTIBIOTIC MED. & CLIN. THERAPY 6:275, 1959 (Lubowe, I. I.) "valuable relief from the pruritus. . . ."

"91%" EFFECTIVENESS RATE IN RESPIRATORY ALLERGIES / NEW YORK STATE J. MED. 59:3060, 1959 (Fuchs, A. M., and Maurer, M. L.) ". . . an outstanding antihistaminic agent for the symptomatic relief of respiratory allergies . . ."

Your literature search would turn up many more references to Dimetane than space here permits. There are papers from distinguished medical centers, investigators, specialists and general practitioners describing the safe efficacy—the easy practicality—of Dimetane in urticaria, allergic rhinitis, eczema, hives, and in almost the entire range of common allergies. Reprints are available. **SUPPLIED:** ORAL: Tablets (4 mg.) or Extentabs® (12 mg.), bottles of 100, 500. Elixir (2 mg./5 cc.), bottles of 1 pint. INJECTABLE: Dimetane-Ten (10 mg./cc.) in 1 cc. ampuls, boxes of 6. Dimetane-100 (100 mg./cc.), 2 cc. size multiple dose vials in boxes of 1.

(PARABROMDYLAMINE MALEATE)

Dimetane® Robins

A. H. ROBINS CO., INC., RICHMOND 20, VIRGINIA
ETHICAL PHARMACEUTICALS OF MERIT SINCE 1878

in edema or

▪ more doctors are prescribing –
▪ more patients are receiving the benefits of –
▪ more clinical evidence exists for –

in congestive failure

"Chlorothiazide was given to 16 patients for a total of 295 patient-treatment days." "Chlorothiazide is a safe, oral diuretic with a clinical effect equal to or greater than a parenteral mercurial." Harvey, S. D. and DeGraff, A. C.: N. Y. State J. Med., **59**:1769, (May 1) 1959.

in hypertension

". . . our program has been one of polypharmacy in which we attempt to deplete body sodium with chlorothiazide. This drug is continued indefinitely as background medication for all antihypertensive drugs." Moyer, J. H.: Am. J. Cardiology, **3**:199, (Feb.) 1959.

in premenstrual edema

"Chlorothiazide is an excellent agent for relief of swelling and breast soreness associated with the premenstrual tension syndrome, since all patients [50] with these complaints were completely relieved." Keyes, J. W. and Berlacher, F. J.: J.A.M.A., **169**:109, (Jan. 10) 1959.

DOSAGE: Edema—One or two 500 mg. tablets DIURIL once or twice a day. Hypertension—One 250 mg. tablet DIURIL twice a day to one 500 mg. tablet DIURIL three times a day.

SUPPLIED: 250 mg. and 500 mg. scored tablets DIURIL (chlorothiazide) in bottles of 100 and 1,000.

DIURIL *is a trademark of Merck & Co., INC.*

Additional information is available to the physician on request.

hypertension

DIURIL®

(CHLOROTHIAZIDE)

than for all other diuretic-antihypertensives combined!

in edema of pregnancy

"One hundred patients were treated with oral chlorothiazide." "In the presence of clinically detectable edema, the agent was universally effective." "Chlorothiazide is at present the most effective oral diuretic in pregnancy." Landesman, R., Ollstein, R. N. and Quinton, E. J.: N. Y. State J. Med., **59**:66, (Jan. 1) 1959.

in cirrhosis with ascites

"All three of the patients with Laennec's cirrhosis, ascites and edema had a favorable response, with a mean weight loss of 8 lbs., during the five-day treatment period with a slight decrease in edema." Castle, C. N., Conrad, J. K. and Hecht, H. H.: Arch. Int. Med., **103**:415, (March) 1959.

in renal edema

"In a study of 10 patients with the nephrotic syndrome associated with various types of renal disease, orally administered chlorothiazide was a successful, and sometimes dramatic, diuretic agent." Burch, G. E. and White, M. A., Jr.: Arch. Int. Med., **103**:369, (March) 1959.

MSD MERCK SHARP & DOHME
Division of Merck & Co., Inc., Philadelphia 1, Pa.

AN AMES CLINIQUICK®

CLINICAL BRIEFS FOR MODERN PRACTICE

WHAT SHOULD YOU LOOK FOR IN A LABORATORY TEST?

THEN LOOK FOR AMES "DIP-AND-READ" REAGENT STRIP URINE TESTS

ALBUSTIX®
for proteinuria

CLINISTIX®
for glycosuria

URISTIX®
for proteinuria and glycosuria

COMBISTIX®
for proteinuria, glycosuria and pH

KETOSTIX®
for ketonuria

PHENISTIX™
for phenylketonuria

standardized color scales...consistent results day after day, test after test
optimal sensitivity...detect clinically significant levels
timesaving...*no filtration or centrifuging* of turbid urines; results in seconds
economical...no extra equipment; up to 3 tests per "dip"
completely disposable...no cleanup afterward

83960

AMES
COMPANY, INC
Elkhart • Indiana
Toronto • Canada

Anturan™

brand of sulfinpyrazone

halting the progress of gout

by potent uricosuric action

By vastly increasing the excretion of uric acid Anturan directly counterbalances the basic metabolic defect in gout.

Clinical experience shows that Anturan: Prevents new tophus formation—causes absorption of pre-existing tophi.[1,2]

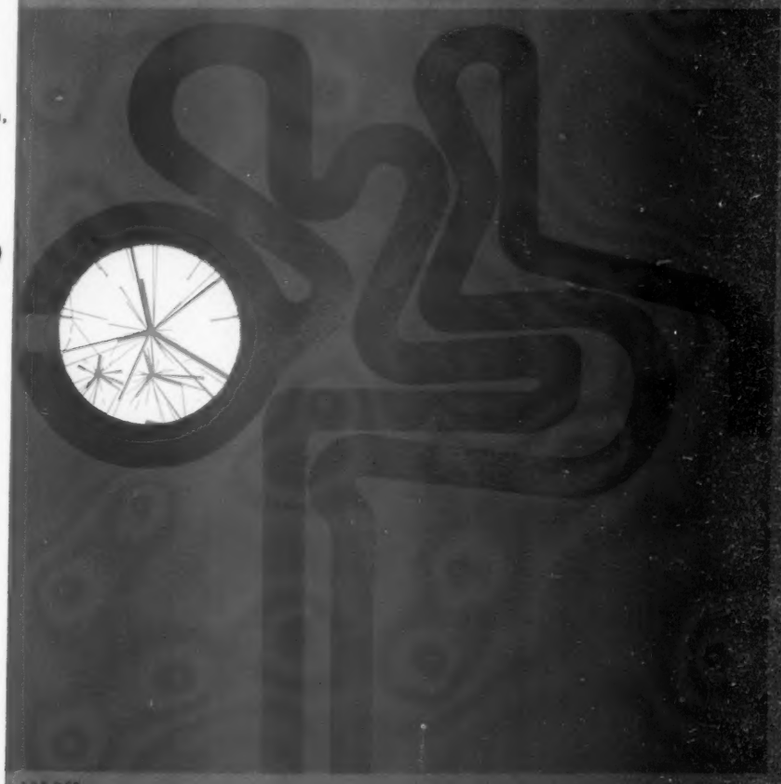
Reduces the incidence and severity of acute attacks after the first few weeks of treatment.[3,4]

Relieves interval pain[2,4]—reduces joint swelling[3]—improves mobility.[2]

References:
1. Yü, T. F., Burns, J. J., and Gutman, A. B.: Arth. & Rheumat. *1*:532, 1958. 2. Gutman, A. B., and Yü, T. F.: Bull. N. Y. Acad. Med. *34*:287, 1958. 3. Kersley, G. D., Cook, E. R., and Tovey, D. C. J.: Ann. Rheumat. Dis. *17*:326, 1958. 4. Ogryzlo, M. A., and Harrison, J.: Ann. Rheumat. Dis. *16*:425, 1957.

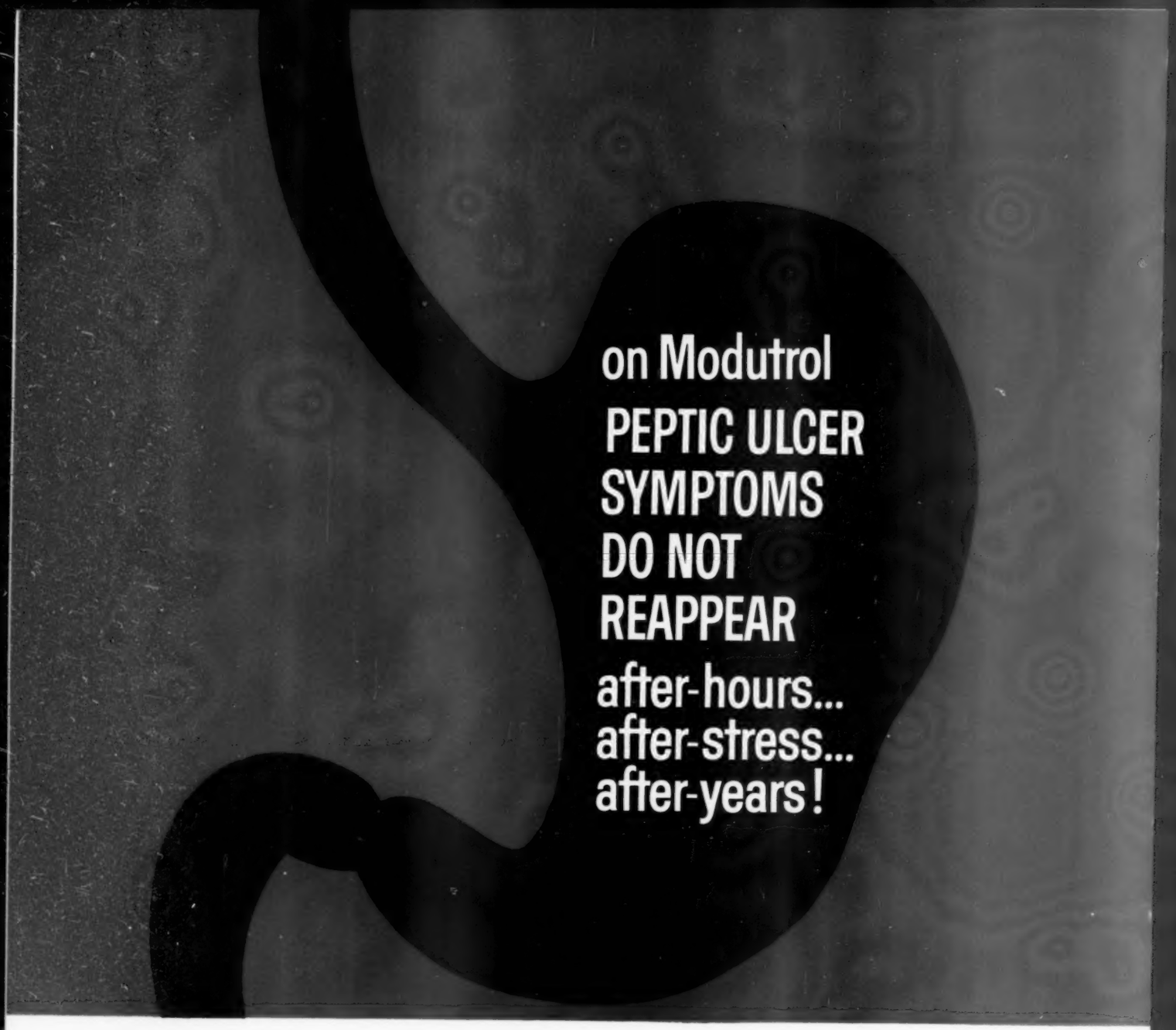
AnturanT.M., brand of sulfinpyrazone: scored tablets of 100 mg. in bottle of 100.
Detailed Literature on Request.

Geigy, Ardsley, New York



Geigy

AN-860



Modutrol allows complete and *lasting freedom* from symptoms—without dietary restrictions. Of all agents tested, only Modutrol achieved the three rigid objectives for success in peptic ulcer therapy: relief of symptoms, healing of ulcer and prevention of recurrences or complications. Moreover, Modutrol met these criteria in over 96 per cent of all patients tested.[1]

Psychophysiologic Medication To Combat A "Psychovisceral Process"

Therapeutic efficacy of Modutrol is enhanced by its psycho-active component, Sycotrol—proved clinically to be not only more effective than either sedatives or tranquilizers, but ideally suited for ambulatory patients because they do not experience commonly encountered side effects of depression and habituation. Sycotrol, a psychotropic agent with antiphobic properties, acts against fears and anxieties that find outlets in visceral manifestations. Modutrol combines the psycho-active agent with preferred antacid and anticholinergic therapy to provide total management of the disorder.

FORMULA: Each Modutrol tablet contains: Sycotrol (pipethanate hydrochloride) 2 mg., scopolamine methylnitrate 1 mg., magnesium hydroxide 200 mg., aluminum hydroxide 200 mg.

DOSAGE: One tablet 3 or 4 times daily.

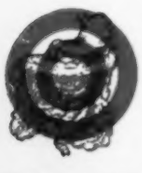
SUPPLIED: Bottles of 50 and 100 tablets.

CONTRAINDICATIONS: Contraindicated in glaucoma because of its anticholinergic components.

1. Rosenblum, L. A.: Report, Symposium on Peptic Ulcer, University of Vermont School of Medicine, September 24, 1959.

Also available: Sycotrol tablets 3 mg. Bottles of 100 tablets.

R&C REED & CARNRICK *Kenilworth, New Jersey*



Psycho-physiologic Management

MODUTROL®

When the Target Organ of Fear-anxieties is the G.I. Tract and Peptic Ulcer Results.

announcing a major event in anticoagulant therapy...

Schering

Certified—before introduction—by 5 years of clinical experience and published reports in the U.S.A., Canada and Great Britain.

Miradon®

anisindione

new oral prothrombin depressant

control at every stage of anticoagulant therapy rapidity of induction and recovery time predictability of initial and maintenance dosages stability of therapeutic prothrombin levels during maintenance therapy reversibility of anticoagulant effect with vitamin K_1 preparations... rapid return to therapeutic levels on remedication

Well tolerated and relatively nontoxic no nausea and vomiting, proteinuria, agranulocytosis or leukopenia yet observed —chromaturia infrequent and transient.

Single daily dose convenience

Packaging—MIRADON Tablets, 50 mg., bottle of 100.

For complete information on indications, dosage, precautions, and contraindications consult the Schering Statement of Directions.

5-435

specific treatment for arthritic joints

intra-articular/intrasynovial/intrabursal instillation

□ **good to excellent response in a vast majority of patients**—"Low doses...provided rapid and effective relief...in almost all of the 157 patients treated..."[1] "...appeared to be superior as an intra-articular injectable substance to anything hitherto available."[2]

□ **provides sustained, long lasting benefits**—In 28 out of 34 patients,"...complete relief was provided by a single injection...the relief lasting for an average of more than 2.5 months."[2]

□ **rapid relief of pain, swelling, and improved range of motion**—"Pain was relieved in 3 or 4 days, or less..."[3] "...marked improvement in range of motion occurred in all of these patients."[3] "...more potent, milligram for milligram, than other injectable corticosteroids."[4]

□ **undesirable side reactions outstandingly rare**—"...appears to be a safe, potent, and effective preparation..."[5] "...tolerated as well as or better than hydrocortisone or prednisolone."[6]

Dosage: usual doses—2.5 to 5.0 mg. for smaller joints; 5.0 to 15.0 mg. for larger joints. *Side Effects:* outstandingly rare; although systemic effects do not ordinarily occur with Kenalog Parenteral when the proper techniques and dosages are used, careful clinical supervision is advisable for all patients receiving steroid therapy. *Contraindications:* infections in or near joints—e.g., gonococcal or tuberculous arthritis. *Supply:* a sterile aqueous suspension in 5 cc. vials, each cc. providing 10 mg. triamcinolone acetonide. *References:* 1. Sperling, I. L.: Clinical Research Notes vol. 3, No. 1 (Jan.) 1960. 2. Steinberg, C. L.: op. cit. 3. Urist, M. R.: op. cit. 4. Meltzer, L. E.: op. cit. 5. Schwartz, S.: op. cit. 6. Felts, W. R.: op. cit.

Among 363 patients treated with Kenalog Parenteral, 315 (86.7%) obtained complete relief or were markedly improved.[1-6]

new **Kenalog Parenteral**

Squibb Triamcinolone Acetonide Aqueous Suspension

'KENALOG'® IS A SQUIBB TRADEMARK.

SQUIBB

Squibb Quality—the Priceless Ingredient

No scurrying sheep, no pounded pillows, no worried waiting for that first yawn, with Placidyl. Just restful slumber, gentle as eiderdown and nearly as quick as a wink. Prompt-acting Placidyl's both effective and non-barbiturate. Try it and see.

ABBOTT

© 1960, ABBOTT LABORATORIES CO4047

Placidyl® nudges your patient to sleep

(Ethchlorvynol, Abbott)

Geriactive with Gerilets®

Geriatric Supportive Formula, Abbott

A FULL RANGE OF DIETARY AND THERAPEUTIC SUPPORT FOR OLDER PATIENTS

B-Complex Vitamins

Thiamine Mononitrate	5 mg.
Riboflavin	5 mg.
Pyridoxine Hydrochloride	1 mg.
Nicotinamide	20 mg.
Calcium Pantothenate	5 mg.

Oil Soluble Vitamins

Vitamin A	1.5 mg. (5000 units)
Vitamin D	12.5 mcg. (500 units)
Vitamin E	10 Int. units

Hematopoietic Factors

Vitamin B_{12} with Intrinsic Factor Concentrate, ½ U.S.P. Unit (oral)	
Ferrous Sulfate, U.S.P. *(Elemental Iron—15 mg.)*	75 mg.
Folic Acid	0.25 mg.

Capillary Stability

Ascorbic Acid	50 mg.
Quertine® *(Quercetin, Abbott)*	12.5 mg.

Lipotropic Factors

Betaine Hydrochloride	50 mg.
Inositol	50 mg.

Anti-Depressant

Desoxyn® *(Methamphetamine Hydrochloride, Abbott)*	1 mg.

Hormones

Sulestrex *(Piperazine Estrone Sulfate, Abbott)*	0.3 mg.
Methyltestosterone	2.5 mg.

FILMTAB—FILM-SEALED TABLETS, ABBOTT; U.S. PAT. NO. 2,881,085.

STREAMLINED INTO THE SMALLEST TABLET **OF ITS KIND**

ABBOTT

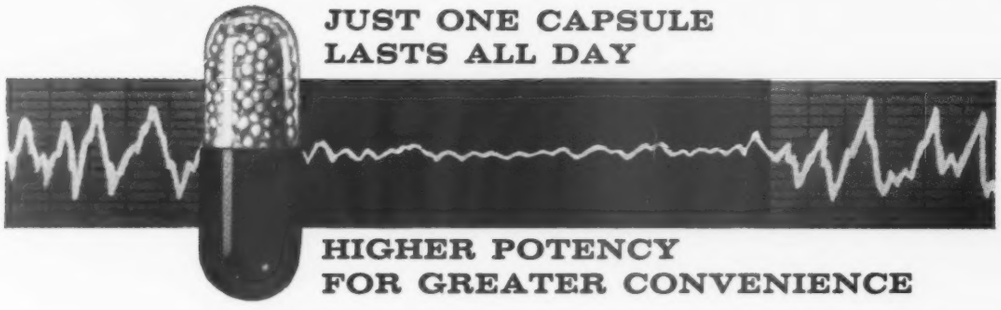
©1960—ABBOTT LABORATORIES, NORTH CHICAGO, ILLINOIS 005048

NEW AND EXCLUSIVE

FOR SUSTAINED TRANQUILIZATION

MILTOWN® (*meprobamate*) now available in 400 mg. continuous release capsules as

Meprospan®-400



- relieves *both* mental and muscular tension without causing depression
- does not impair mental efficiency, motor control, or normal behavior

Usual dosage: One capsule at breakfast, one capsule with evening meal

Available: *Meprospan-400*, each blue capsule contains 400 mg. Miltown (meprobamate)
Meprospan-200, each yellow capsule contains 200 mg. Miltown (meprobamate)
Both potencies in bottles of 30.

WALLACE LABORATORIES, *New Brunswick, N. J.*

CME-8427

©1960—ABBOTT LABORATORIES, NORTH CHICAGO, ILLINOIS 605048

in musculoskeletal disorders

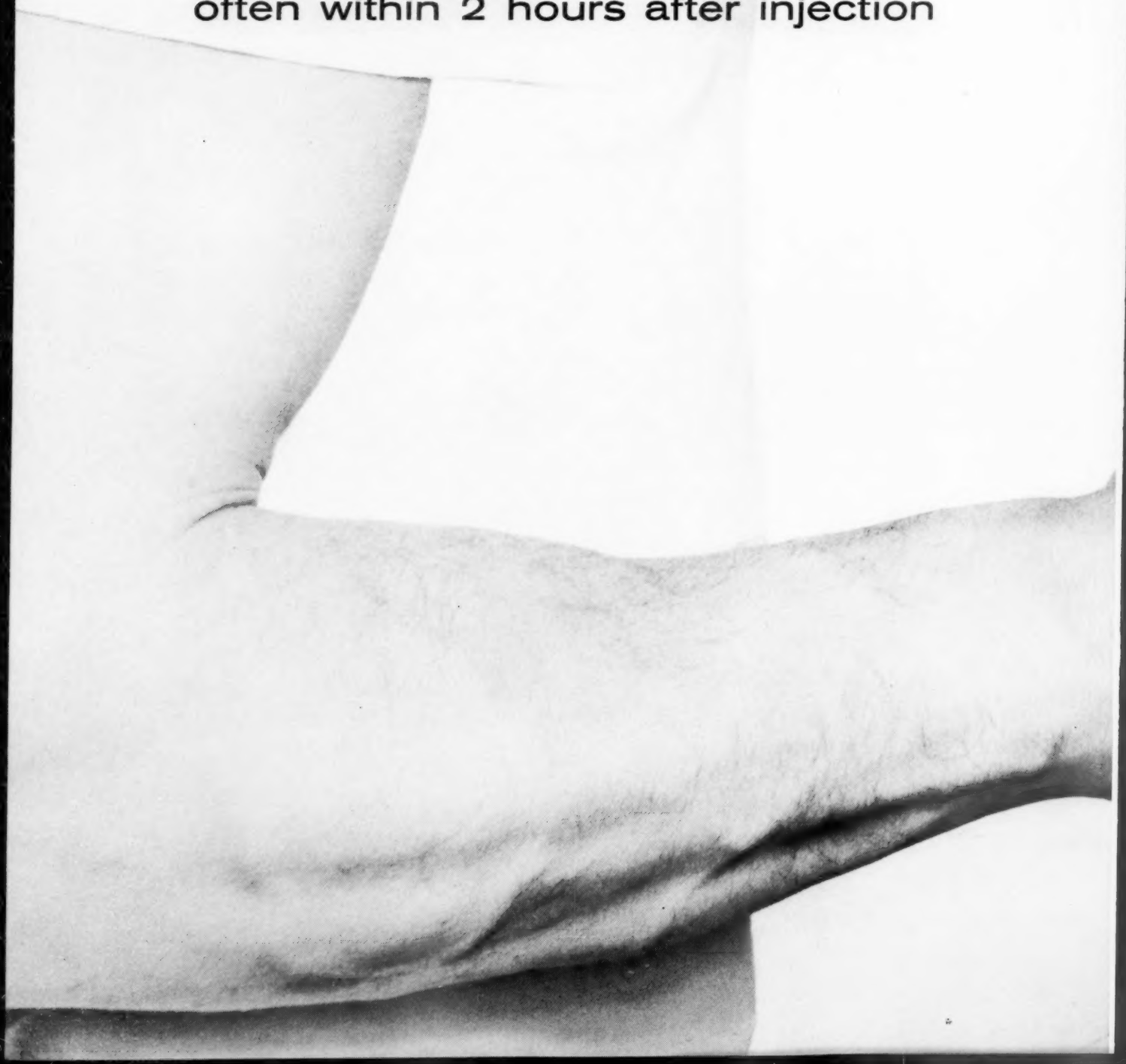
INJECTION

Decadron® PHOSPHATE

DEXAMETHASONE 21-PHOSPHATE

usually controls the acute phase

often within 2 hours after injection



THE DIRECT APPROACH — rapid relief and prolonged effect—complete solubility affords quick diffusion of the therapeutic dose

THE DIRECT APPROACH — mg. for mg. the most active steroid

THE DIRECT APPROACH — passes easily through small-bore needles

THE DIRECT APPROACH — needs no reconstitution—no refrigeration

Injection DECADRON Phosphate can be used in strains and sprains, bursitis, fibrositis, rheumatoid arthritis, traumatic arthritis, acute gouty arthritis, osteoarthritis, osteochondritis, "trigger" points (localized painful areas in muscles), tendinitis, whiplash injuries (acute), and muscle trauma.

Caution: Steroids should not be given in the presence of tuberculosis, chronic nephritis, acute psychosis, peptic ulcer, or ocular herpes simplex.

Note: Do not inject into intervertebral joints.

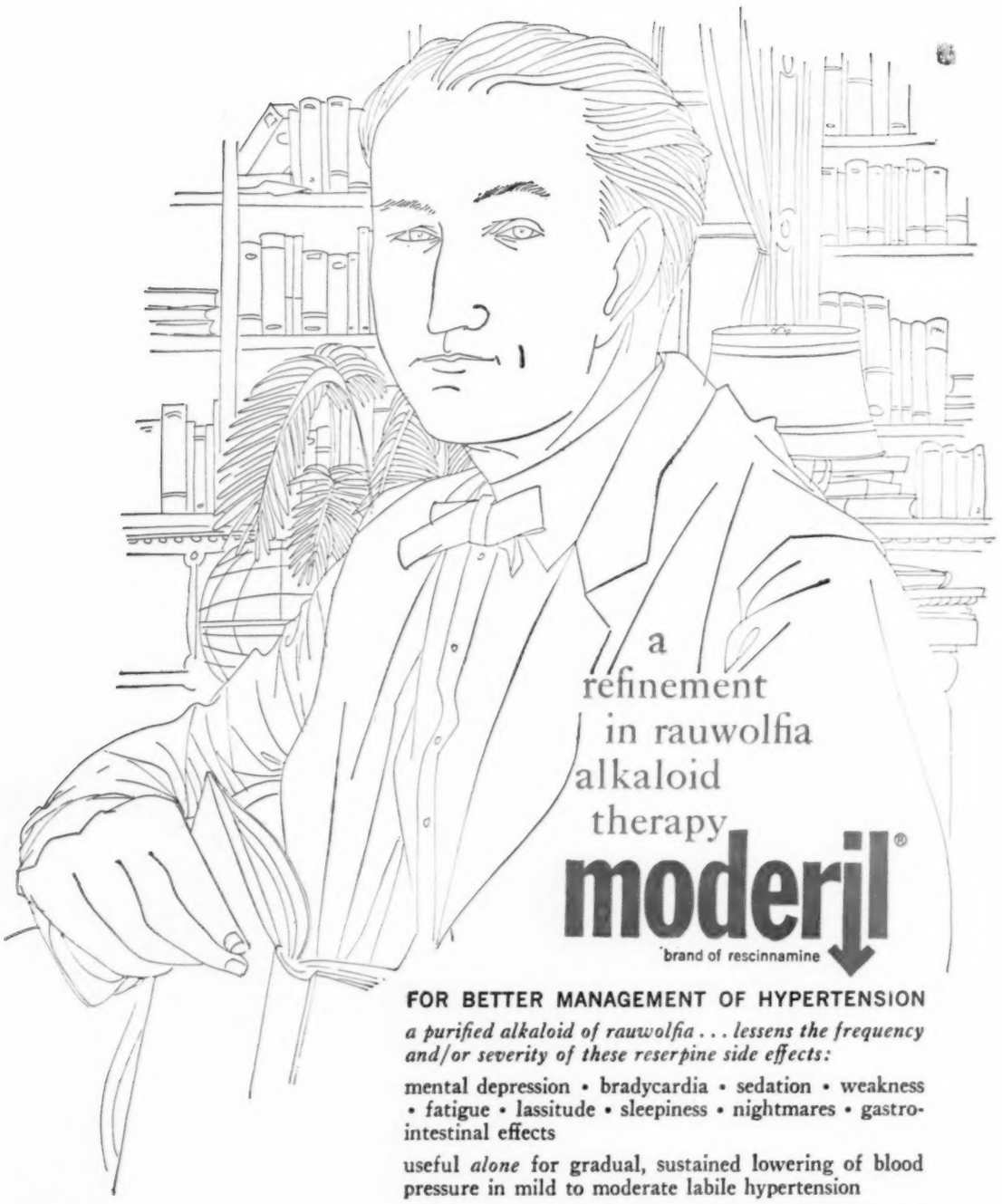
DOSAGE AND ADMINISTRATION:
Injection DECADRON Phosphate is ready for immediate use intramuscularly or intra-articularly.
Dosage varies from 0.4 to 6 mg. or more depending on the nature and severity of the condition.

SUPPLIED:
5 cc. vials with 4 mg. dexamethasone 21-phosphate as the disodium salt per cc.

Additional information on Injection DECADRON Phosphate is available at your request.

DECADRON is a trademark of Merck & Co., INC.

MSD MERCK SHARP & DOHME ◦ Division of Merck & Co., INC. ◦ West Point, Pa.



a refinement in rauwolfia alkaloid therapy

moderil®

*brand of rescinnamine

FOR BETTER MANAGEMENT OF HYPERTENSION

a purified alkaloid of rauwolfia . . . lessens the frequency and/or severity of these reserpine side effects:

mental depression • bradycardia • sedation • weakness • fatigue • lassitude • sleepiness • nightmares • gastrointestinal effects

useful *alone* for gradual, sustained lowering of blood pressure in mild to moderate labile hypertension

useful *as an adjunct* to other types of antihypertensive agents, permitting their use in lower, better tolerated dosage

Professional information available on request

PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. Brooklyn 6, N.Y. Pfizer *Science for the world's well-being™*

Advertisers' Product Index

May, 1960

Abbott Laboratories
Gerilets* ... 156
Iberol* ... 26
Nembutal* ... 61
Panwarfin ... 110–111
Placidyl* ... 155
Selsun* ... 25
Spontin* ... 55

Ames Company, Inc.
Clinitest* ... 10
Diagnostics* ... 150

American Sterilizer
Double Cabinet ... 78

Armour Pharmaceutical Company
Thyroid* ... 88

Ayerst
PMB-200* ... 52
Timovan ... 120–121

Burroughs Wellcome & Co., Inc.
Migral* ... 122

Cambridge Instrument Co., Inc.
Pulmonary Function Tester ... 104

Ciba Pharmaceutical Products, Inc.
Dianabol* ... 115
Esidrix* ... 86
Serpasil* ... 102
Singoserp* ... 12–13, 32, Fourth Cover
Singoserp-Esidrix* ... 18

Eaton Laboratories
Altafur* ... 22
Tricofuron* ... 91

Endo Laboratories
Coumadin* ... 69
Percodan* ... 135

Forrest Company
Tri-Synar ... 94

Fougera, E., & Co.
Diasol* ... 113

Geigy Company
Anturan* ... 151
Butazolidin* ... 97
Preludin* ... 83
Sterosan* ... 33

Hyland Laboratories, Inc.
TA-Test ... 44

Irwin, Neisler & Co.
Analexin* ... 107–108–109

Ives-Cameron Company
Isordil* ... 65

Lederle Laboratories, A Division of American Cyanamid Company
Aristocort* ... 16–17
Stresscaps* ... 95

Leeming, Thos., & Company, Inc.
Clarin* ... 75

Lilly, Eli, and Company
Darvon* ... 72

Maltbie Laboratories
Dornwal* ... 42

Massengill, The S. E., Company
Livitamin* ... 35–36
Obedrin* ... 89–90

McNeil Laboratories, Inc.
Syndrox* ... 11
Tylenol* ... 87

Mead Johnson & Co.
Metrecal* ... 56–57

Merck Sharp & Dohme, Div. Merck & Co., Inc.
Benemid* ... 92–93
Cremomycin* ... 24
Decadron* ... 40–41
Diupres* ... 8–9
Diuril* ... 148–149
Hydeltra* ... 62
Hydro-Diuril* ... 70–71
Injection Decadron* ... 158–159
Mephyton* ... 100–101
Pentazets* ... 123

Organon, Inc.
Nugestoral* ... 46

Parke, Davis & Company
Eldec* ... 138–139
Myadec* ... 99
Norlutin* ... 43

Pfizer Laboratories Div., Chas. Pfizer & Co., Inc.
Ataraxoid* ... 98
Bonine* ... 58–59
Cosa-Terramycin* ... 19
Cosa-Terrabon* ... 143
Daricon* ... 14
Diabinese* ... 30
Moderil* ... 160
Niamid* ... 130
Vistaril* ... 114

*** Complete description of starred drugs will be found in MODERN DRUGS and THE MODERN DRUG ENCYCLOPEDIA.**

Pitman-Moore Company
Emdee Margarine 53–54

Reed-Carnrick
Modutrol* 152

Riker Laboratories, Inc.
Medihaler-Ergotamine* ..... Third Cover
Rauwiloid* 23

Robins, A. H., Company, Inc.
Ambar* 145
Dimetane* 146–147
Donnazyme* 81
Phenaphen* 133

Roche Laboratories, Div. Hoffmann-La Roche, Inc.
Azo Gantrisin* 105
Alurate* 31
Librium* 84–85
Madribon* 28–29
Noludar* 117
Tigan* 38–39

Roerig, J. B., & Co.
Antivert* 140
Atarax* 106
Cartrax* 37
Maxipen* 50–51

Sandoz Pharmaceuticals, Div. Sandoz, Inc.
Cafergot* 79
Mellaril* 63–64

Schering Corporation
Alpen 76–77
Miradon* 153
Naqua* 45, 47, 49
Polanil* 103
Rela* 20–21

Schieffelin & Company, Inc.
Danilone* 141

Searle, G. D., & Co.
Aldactone* 73

Sherman Laboratories
Elixophyllin* 27

Squibb & Sons, E. R., Div. Mathieson Chemical Corp.
Kenalog Parenteral* 154
Kenalog Spray* 2
Naturetin-K* 124
Noctec* 164
Pentids* 34, 116, 144
Vesprin* 82

Sunkist Growers
Citrus Bioflavonoids 112–113

Upjohn, The, Company
Medaprin* 131
Medrol* 80
Orinase* 66

Wallace Laboratories
Deprol* 118–119
Meprospan* 157
Miltown* 6, 60
Milpath* 74
Miltrate* 96
Soma* 142

Warner-Chilcott Laboratories
Peritrate* 1

White Laboratories, Inc.
Permitil* 67

Winthrop Laboratories
Alevaire* 4
Trancopal* 125–126–127–128

Wyeth Laboratories
Aludrox* 48
Equanil* 15
Equanitrate* 68
Oxaine* 136–137
Sparine* 163

Wynn Pharmacal Corporation
Quinaglute* 132

*** Complete description of starred drugs will be found in MODERN DRUGS and THE MODERN DRUG ENCYCLOPEDIA.**

ADVERTISING REPRESENTATIVES

New York
P. D. Brewer, H. S. Schultz, A. K. Detwiller
MUrray Hill 3-2980

Chicago
R. H. Andrew, C. P. Haffner
WAbash 2-7738

when emotional turbulence threatens medical or surgical care

Fear, agitation, and resistance often hinder medical diagnosis and treatment.

SPARINE alleviates agitation, overcomes resistance, placates fears.

In addition to calming the patient, SPARINE controls other interfering symptoms: nausea, vomiting, and hiccups.

Wyeth Laboratories, Philadelphia 1, Pa.

Sparine®

HYDROCHLORIDE

Promazine Hydrochloride, Wyeth

INJECTION
TABLETS
SYRUP

100 ANNIVERSARY
Wyeth

A Century of Service to Medicine

Squibb Chloral Hydrate

noctec :and the rest is easy!

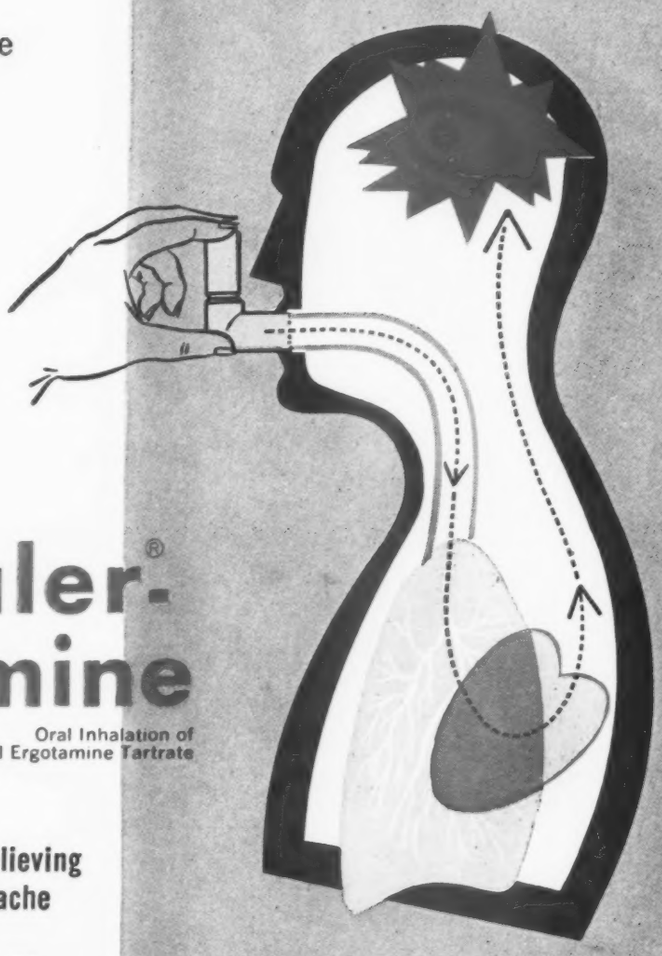
Noctec (Squibb Chloral Hydrate) invites gentle repose — swiftly, safely — soon followed by refreshing sleep best described as "physiologic" in nature. Virtually free of side effects (including preliminary excitement or resultant "hangover" commonly observed with barbiturates), Noctec offers reliable, conservative sleep therapy for patients of all ages. In therapeutic doses, Noctec may also be prescribed when heart disease or other illness is present • in psychiatric complications • during first stage of labor • for pre- and postoperative sedation. *Adults:* 1 or 2 7½ gr. capsules or 1 or 2 teaspoonfuls of solution 15 to 30 minutes before bedtime. *Children:* 1 or 2 3¾ gr. capsules or ¼ to 1 teaspoonful of solution 15 to 30 minutes before bedtime. *Supply:* capsules, 7½ and 3¾ gr.; solution, 7½ gr. per 5 cc. teaspoonful.

SQUIBB
Squibb Quality –
the Priceless Ingredient

NOCTEC® IS A SQUIBB TRADEMARK

on-the-go relief from recurrent throbbing headaches

including migraine syndromes, other vascular headaches, histaminic cephalalgia, and occipital neuralgia



Medihaler®-Ergotamine

Oral Inhalation of
Micronized Ergotamine Tartrate

Fastest overall method for relieving recurrent throbbing headache

Approximates speed and predictability of relief following ergotamine injection.

Eliminates delay in treatment...Medihaler travels *with* the patient...ready and in use in 5 seconds!

"In a series of over 300 episodes of vascular headache in 41 patients 'Medihaler'-Ergotamine was effective in about 70%."

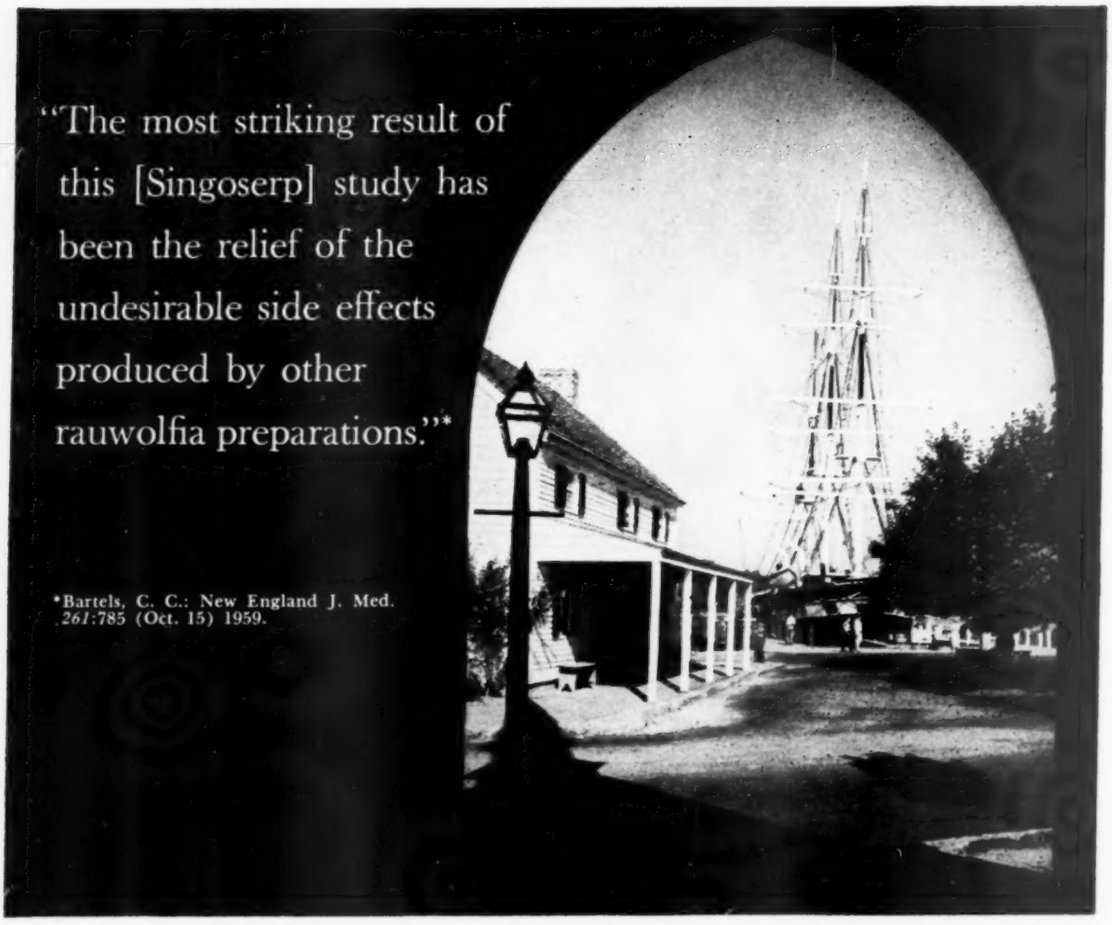
Graham, J.R.: Faulkner Hospital, Jamaica Plains, Boston.

Dosage: A single inhalation at onset of headache. Additional inhalations should be spaced not less than 5 minutes apart. Not more than 6 inhalations in any 24-hour period.

In 2.5 cc. stainless steel vial (50 doses) with plastic oral adapter. Each depression of metering valve delivers 0.36 mg. ergotamine tartrate self-propelled from the oral adapter.

Riker Northridge, California

from the New England Journal of Medicine:


"The most striking result of this [Singoserp] study has been the relief of the undesirable side effects produced by other rauwolfia preparations."*
*Bartels, C. C.: New England J. Med. 261:785 (Oct. 15) 1959.

results you can confirm in your practice:

"In 24 cases syrosingopine was substituted for the rauwolfia product because of 26 troublesome side effects; these symptoms were relieved in all but 3 patients."*

Side Effects	Incidence with Prior Rauwolfia Agent	Incidence with Singoserp
Depression	11	1
Lethargy or fatigue	5	0
Nasal congestion	7	0
Gastrointestinal disturbances	2	2
Conjunctivitis	1	0

(Adapted from Bartels*)

many hypertensive patients prefer

Singoserp®

(syrosingopine CIBA)

because it lowers their blood pressure without rauwolfia side effects

Tablets, 1 mg. (white, scored); bottles of 100.

CIBA
SUMMIT, NEW JERSEY

Complete information available on request.

2/377SNS